Biology and pathogenicity of cereal cyst nematodes on wheat in Ismailia, Egypt

Von der Fakultät für Lebenswissenschaften

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von **Mohamed Hassan Awad Baklawa**

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Mohamed Baklawa *Braunschweig, July 2013*

To my family ……. More than ever.

Families are the compass that guides us.

They are the inspiration to reach great heights.

They are our comfort when we occasionally falter.

Brad Henry

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Name of Candidate	***Mohamed Hassan Awad Baklawa***
Title of Thesis	*Biology and pathogenicity of cereal cyst nematodes on wheat in Ismailia, Egypt*
Degree	*Ph.D.*
University	*Technical University Braunschweig*
Place	*Braunschweig, Germany*
Language	*English*

SUMMARY

A survey on cereal cyst nematodes (CCN) was carried out in wheat production areas in Ismailia province, Egypt. CCN were found in five out of seven regions in Ismailia. The highest incidence of CCN was found at El Shark location (West Sinai). All Egyptian populations were identified as *Heterodera avenae* based on morphometrics of cyst vulval cones and second stage juveniles. The Egyptian populations of *H. avenae* were related to *H. avenae* populations belonging to Type B based on ITS-RFLP patterns generated by restriction enzymes while a German population (Grafenreuth) of *H. avenae* was found related to Type A. Analyses of ITS region sequences confirmed the species identification of the Egyptian populations. They were clustered with *H. avenae* populations from Iran, Saudi Arabia, India and China. The hatching pattern of the Egyptian populations of *H. avenae* was similar to the Mediterranean ecotype with winter activity while the German population was similar to the Northern ecotype with spring activity. The reduction in grain yield of the Egyptian wheat cultivars by *H. avenae* ranged between 15 - 42% under greenhouse conditions. The Egyptian populations of *H. avenae* have the same virulence as pathotype Ha13 while the German population could be assigned to pathotype Ha11. The Egyptian wheat cultivar 'Sakha 93' could be classified as tolerant to *H. avenae* populations under greenhouse conditions. Control strategies such as early planting and rotation that are effective against the Mediterranean ecotype of *H. avenae* in several countries should be developed against the Egyptian populations of *H. avenae*.

Name des Kandidaten	***Mohamed Hassan Awad Baklawa***
Titel der Dissertation	*Biologie und Pathogenität von Getreide zystennematoden an Weizen in Ismailia, Ägypten*
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Sprache	*Englisch*

ZUSAMMENFASSUNG

In Weizenanbaugebieten der ägyptischen Provinz Ismailia wurde eine Studie über Getreidezystennematoden (CCN) durchgeführt. In fünf von sieben Regionen in Ismailia wurden CCN gefunden, am häufigsten in El Shark (West Sinai). Die ägyptischen Populationen wurden anhand der Morphometrie der Zysten und Larven als *Heterodera avenae* identifiziert. Mittels Restriktionsenzymen erzeugte ITS-RFLP-Muster zeigten, dass die ägyptischen Populationen verwandt mit *H. avenae*-Populationen des Typs B sind und dass eine deutsche *H. avenae*-Population (Grafenreuth) verwandt ist mit Typ A. Sequenzanalysen der ITS-Regionen bestätigten die Identifikation der ägyptischen Population. Anhand der Sequenzanalysen konnten die ägyptischen *H. avenae*-Populationen mit Populationen aus dem Iran, aus Saudi-Arabien, Indien und China gruppiert werden. Das Schlupfverhalten der ägyptischen Populationen von *H. avenae* ähnelte dem Mittelmeer-Ökotyp mit Winteraktivität, während die deutsche Population dem Nord-Ökotyp mit Frühlingsaktivität ähnelte. Der Kornertrag der ägyptischen Weizensorten wurde durch *H. avenae* um 15 bis 42 % reduziert. Außerdem scheinen *H. avenae*-Populationen aus Ägypten die gleichen Virulenzen wie der Pathotyp Ha13 zu haben, während die deutsche Population dem Ha11-Pathotyp ähnelt. In dieser Studie konnte die ägyptische Weizensorte ‘Sakha 93‘ unter Gewächshausbedingungen als tolerant gegen Populationen von *H. avenae* eingestuft werden. Maßnahmen wie frühes Pflanzen und Fruchtfolgen, die in mehreren Ländern effektiv gegen den Mittelmeer-Ökotyp von *H. avenae* eingesetzt werden, könnten auch für die ägyptischen Populationen von *H. avenae* entwickelt werden.

CHAPTER 1

General Introduction and thesis outlines

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Wheat (*Triticum aestivum L.*)

Cereals are grown on more land area than any other food crop. In 2010, world production of wheat was 651 million tons, making it the third most-produced cereal after maize (844 million tons) and rice (672 million tons) **(FAO, 2010)**. The largest importers of wheat in 2009 were, in order of imported quantities: Egypt, European Union, Brazil, Indonesia, Algeria and Japan **(FAO, 2009)**. Egypt is the world's largest wheat importer, importing an estimated 11.5 million tons in the 2011/12 (July/June) marketing year. In previous years on average 10 million tons of wheat were imported **(FAO 2012)**.

Wheat cultivation and production in Egypt

- Climate

Egypt occupies the north-east corner of Africa and lies between latitudes 22°N and 32°N and longitudes 25°E and 36°E. Most of the country has a hot sub-tropical desert climate. Winters are without frost, but sufficiently cool for wheat. Rainfall is negligible. No crop can be grown in this climate without irrigation. In Upper Egypt, warmer mean daily temperatures during winter negatively affect wheat yields. In Middle Egypt and the Nile Delta, winter temperatures are suitable for wheat. The mean daily temperature during the wheat growing period at Giza (Middle Egypt) is 15.7°C and at Mansoura (Delta) 16.4°C. By comparison, the mean daily temperature at Aswan (Upper Egypt) during the same period is 21.4°C. As a result, average wheat yields in Upper Egypt are about half of those obtained in Middle Egypt and the Delta **(FAO 2012)**.

- Wheat cultivation

Wheat occupies about one-third of the total winter crop area. It is cultivated on about 1.3 million hectares and the trend is for increase due to its importance in food security. New wheat plantations are established in newly reclaimed lands. The main Egyptian varieties are Sakha 69, 93, 94, 61, Gemmeza 5, 7, 9, 10, Giza 168, 170, Misr ½

(durum), Beni Sewif 3 (durum), Seds 1, 4, 10 and Sohage 3 (durum). Certain varieties carry special traits. For example, Gemmeza 5, 7, 9, Giza 168, 170 and Sakha 93 are resistant to rust. Sakha 93 and Seds 1 are highly tolerant to water and soil salinity; Giza 165 and Seds 10 are resistant to high temperatures. Seds 4 is early ripening and Sahel 1 is drought tolerant **(FAO 2012)**.

- Wheat production

In Egypt, yearly production of wheat is on average 8.7 million tons. The average yield in Egypt is 6.5 tons/hectare which is much higher than the world average of 2.8 tons/hectare **(FAO 2012)**. Wheat production potential in Egypt can be grouped/attributed to four zones **(Figure 1). Zone 1 (P1)** has high potential for wheat production, **Zone 2(P2)** has moderate potential for wheat production, **Zone 3 (P3)** has low potential for wheat production and **Zone 4(P4)** has no potential for wheat production.

Figure 1. Wheat production potential in Egypt **(FAO 2012)**.

Cereal cyst nematodes

Nematodes are microscopic roundworms that live in many habitats. At least 2500 species of plant-parasitic nematodes have been described, characterized by the presence of a stylet, which is used for the penetration of plant tissue. Most attack roots and underground parts of plants, but some are able to feed on leaves and flowers. Plant-parasitic nematodes are of great economic importance. Because most of them live in the soil, they represent one of the most difficult pests to detect, identify and control **(Stirling *et al.,* 1998)**. Their effects are commonly underestimated by farmers, agronomists and pest management consultants, but it has been estimated that some 10 percent of world crop production is lost as a result of plant nematode damage **(Whitehead, 1998)**.

Although many nematodes have been found associated with small-grain cereals, only a few are considered economically important. Those of importance include: (i) cereal cyst nematodes, *Heterodera spp.*; (ii) root lesion nematodes, *Pratylenchus spp.*; (iii) root knot nematodes, *Meloidogyne spp.*; (iv) seed gall nematode, *Anguina tritici*; and (v) stem nematode, *Ditylenchus dipsaci*.

The cereal cyst nematodes (CCNs) are the most important group of plant parasitic nematodes attacking cereals, including wheat and barley **(Sikora 1987)**. Cereal cyst nematodes are a group of closely related species which have been reported to cause economic yield loss in wheat production systems in several parts of the world including North Africa, West Asia, China, India, Australia, the United States of America and countries in Europe **(Nicol and Rivoal 2008)**.

The species most reported are *Heterodera avenae*, *H. filipjevi* and *H. latipons* **(Rivoal and Cook 1993)** and each species consists of different pathotypes. At least 12 pathotypes have been described for *H. avenae*. Their worldwide distribution, predominance in areas where cereal is grown, and their devastating yield loss rank them as pests affecting the world's food supply.

Distribution

The most reported species, *Heterodera avenae* Wollenweber, was described in the beginning of the 20th century. Description of this species was followed after the middle of this century by those of the Mediterranean *Heterodera latipons*, the north European *Heterodera hordecalis*, the eastern European *Heterodera filipjevi* and several others, to total more than 12 species **(Wouts *et al.,* 1995)**.

The most economically important cereal cyst nematode species *H. avenae*, has been detected in many countries, including Australia, Canada, Israel, South Africa, Japan and most European countries **(Kort, 1972)**, as well as India **(Sharma and Swarup, 1984; Sikora, 1987)** and countries within North Africa and West Asia, including Morocco, Tunisia, Pakistan and Libya **(Sikora, 1987)**, Algeria **(Mokabli *et al.,* 2001)** and Saudi Arabia **(Ibrahim *et al.,* 1999)**. Although its distribution is global, much of the research has been confined to Europe, Canada, Australia and India **(Swarup and Sosa-Moss, 1990)**.

The first record of a species from the *H. avenae* group on wheat in Egypt was by **Ibrahim *et al.,* in 1986**, they found *H. avenae* on barley and wheat in the Nile Delta (EL-Behera governorate). **Ibrahim and Handoo** in **2007** reported the occurrence of *H.avenae* on Egyptian wheat in a survey conducted in Alexandria and El-Behera Governorates in northern Egypt. Despite of these reports, still little is known about the occurrence and distribution of cereal cyst nematode on wheat in Egypt.

Biology and life cycle

The host range of *H. avenae* is restricted to graminaceous plants and has only one generation per year **(Rivoal and Cook, 1993)**. There is sexual dimorphism with the male remaining vermiform, whereas the female becomes lemon-shaped and spends its life inside or attached to the root. The adult white female is clearly visible on roots with the swollen body, about 1 mm across, protruding from the root surface **(Figure 2A)**.

Eggs are retained within the female's body (about 700µm in length), and after the female has died, the body wall hardens to a resistant brown cyst **(Figure 2B)**, which protects the eggs and juveniles **(Figure 2 C&D)**. The eggs within the cyst remain viable for several years **(Kort, 1972)**. The infective juvenile stage **(Figure 2D)** has a vermiform body that is about 550 µm long and 20µm in diameter.

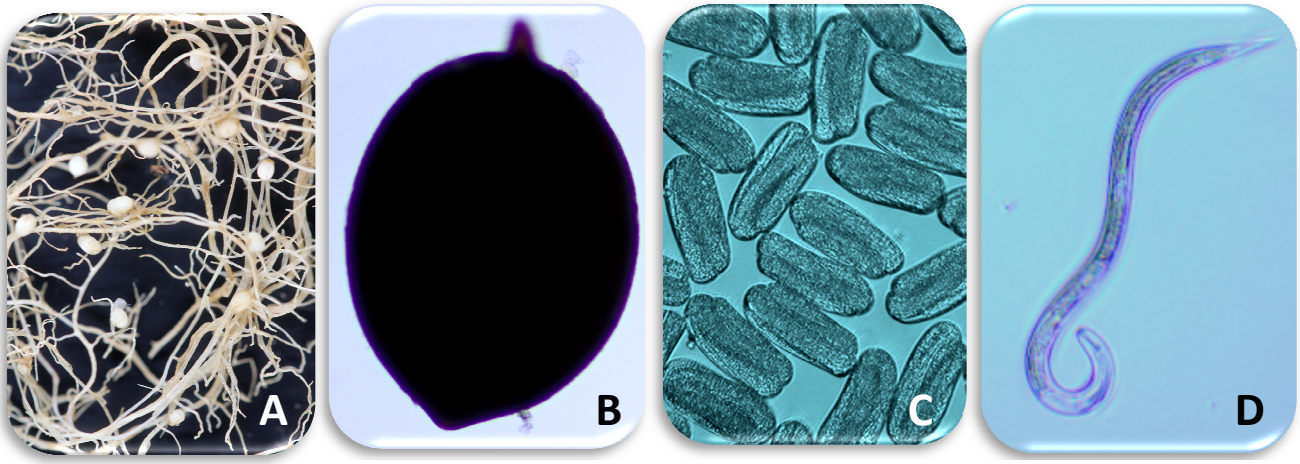

Figure 2. Life stages of *H. avenae*: **(A)** enlarged white female protruding from roots; **(B)** lemon-shaped brown cyst containing hundreds of eggs; **(C)** eggs containing developing infective juvenile stage; **(D)** infective juvenile stage.

Figure 3 shows the life cycle of the cyst nematode *Heterodera* spp. Second stage juveniles penetrate epidermal and cortical cells only at the tips of new roots. They feed and induce the formation of enlarged feeding cells called syncytia. Here they develop, into sedentary bottle-shaped third stage juvenile and rounded fourth stage juveniles. These then develop into either females or males.

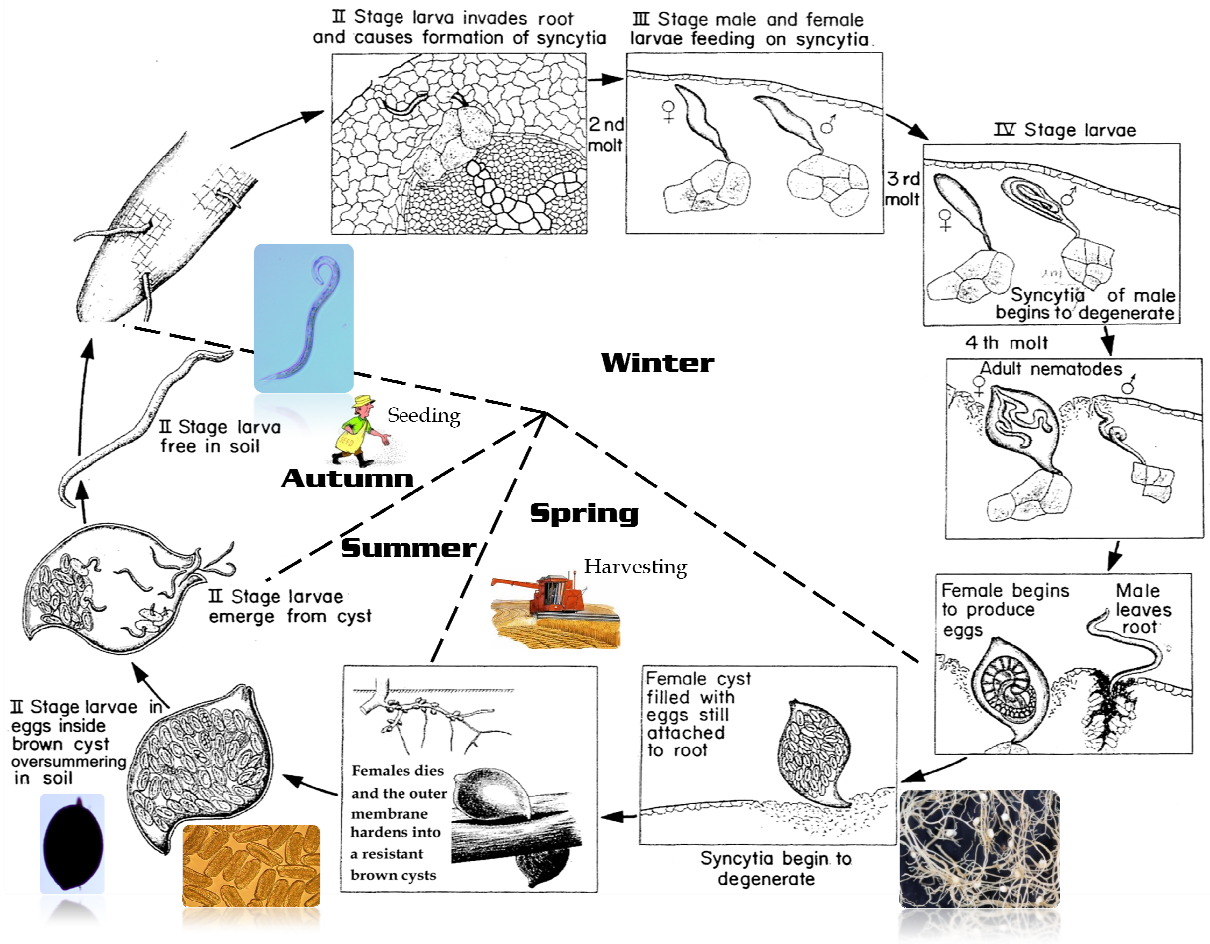

Figure 3. Life cycle of the Mediterranean ecotype of cereal cyst nematode *Heterodera avenae*. **(**modified from **Agrios, 1997)**.

Males remain mobile, but females become embedded in the root tissue and continue to feed from the syncytium. The males fertilize the sedentary females, and the fertilized female bodies become swollen as it fills with eggs. When invaded roots mature and senescence, the female dies and the outer membrane hardens into a resistant brown cyst. The cysts protect eggs and juveniles from any unsuitable conditions for several years **(Smiley and Yan, 2010)**.

Hatching and temperature requirements

The effect of long-term and seasonal temperature variations on the hatching of cereal cyst nematodes has been studied. **Evans and Perry (1976)** described two different hatching behaviors according to the geographical origin of populations: 1- winter activity in Mediterranean climate regions; 2- spring activity in the Northern regions of Europe and America. These two hatching patterns were observed in France **(Rivoal, 1978)**. In a southern population, hatching essentially occurred in winter whereas a northern population released its juveniles in spring. This activity appeared to be due to induction or interruption of two different types of dormancy as a function of seasonal temperature variations.

Evans and Perry (1976) considered that definitions of terms concerned with nematode survival should be based on the cause of arrest in development. They viewed quiescence and diapause as types of dormancy. Quiescence being induced in response to unfavorable conditions and ended by the return of favorable conditions. Diapause being the condition in which development has been arrested and cannot be resumed until specific requirements have been satisfied, even if favorable conditions return.

Quiescence occurs by high temperatures in summer on newly-formed juveniles of the southern populations; it ceases by autumn when temperatures fall below 10°C. In Mediterranean climates, the quiescence takes place when hot dry conditions prevail and is inhibit when temperatures fall and soil moisture increases. For instance, cysts of *H. avenae* in Australia have to survive a hot dry summer before larval emergence begins in late autumn and continues into winter **(Meagher, 1970)**.

Diapause is usually induced by high summer temperatures in the northern populations of *H. avenae*. After exposure to low temperature 2-7°C, juveniles hatched when moved to 10 or 15°C **(Rivoal, 1979)**. **Cotten (1962)** demonstrated that low temperature stimulates hatching of *H. avenae*, and subsequently a minimum period of 8 weeks at low temperature of 5°C has been found necessary for substantial hatching to occur **(Fushtey and Johnson, 1966)**.

The combined action of these types of dormancy and of the temperature variations determined several successive annual cycles for juvenile emergence **(Rivoal, 1983)**. The hatching cycles of the Southern and Northern populations were maintained even after transfer to a climatically different location, which suggests they express adaptation to particular environmental conditions **(Rivoal, 1983)**.

For that reason the term ecotypes was used **(Rivoal, 1982)**. The southern ecotype, would favor survival of populations located in Mediterranean climate, while behavior of the northern population corresponds to the hatching pattern observed in *H. avenae* in a more or less temperate climate.

Identification and characterization

The first illustrated key to the 34 cyst-forming genera and species of Heteroderidae in the western hemisphere was given by **Mulvey and Golden (1983)**. The taxonomy of the *H. avenae* group has been advanced by numerous review papers published by different workers **(Ferris *et al.,* 1989, 1994; Golden, 1986; Robinson *et al.,* 1996; Rumpenhorst *et al.,* 1996; Vovlas, 1985; Wouts and Sturhan, 1995; Wouts *et al.,* 1995)**.

New cysts of *H. avenae* usually have a sub-crystalline layer which is a white flaky layer enclosing the cysts and probably forms by condensation of plant and nematode metabolites between the cuticle of the final stage larvae and that of the adult **(Brown *et al.,* 1971)**. Typically cysts are very dark brown to black when mature.

The word fenestra refers to the thin-walled area on the vulval cone or perineal area of mature cysts. The vulval cone is ambifenestrate and supports the vulval slit **(Figure 4)**. In young cysts the fenestral area is membranous but later decays, leaving a hole in the cyst wall **(Turner and Rowe, 2006)**.

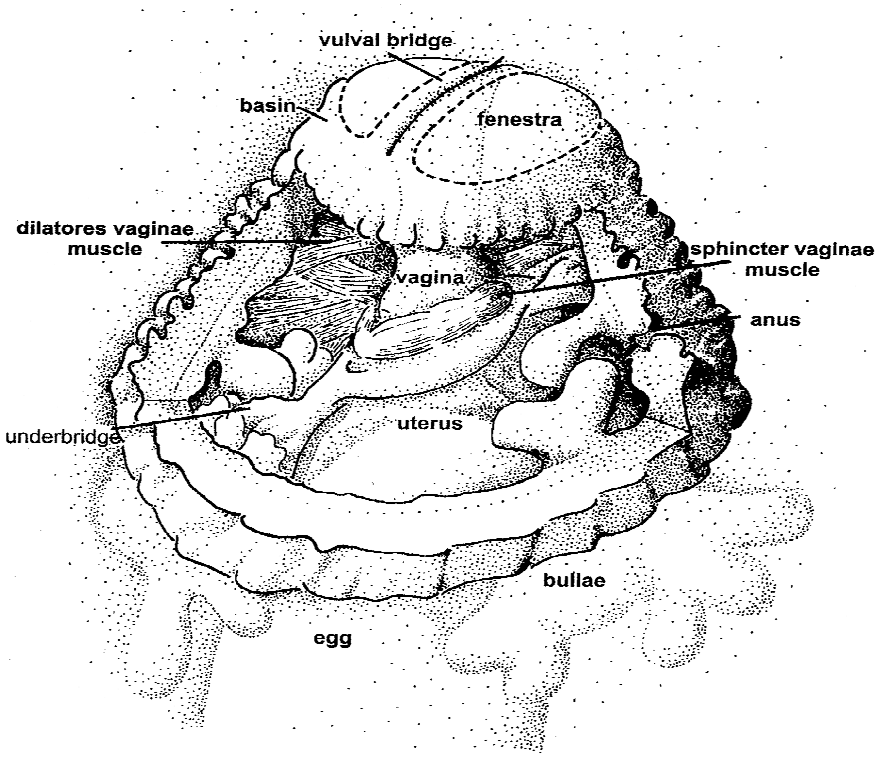

Figure 4. Vulval cone details of cysts of *Heterodera* spp. **(Sharma and Sharma, 1998)**.

There are three main types of fenestration: circumfenestrate, bifenestrate and ambifenestrate. The fenestration is an important feature in identification. The remains of the vagina and musculature can be seen as the bullae, which crowd towards the top of the cone. In the *H. avenae* group it is unusual to find an under bridge; many other species within the *H. avenae* group do have an under bridge, and this is a distinguishing feature. **Figure 5** shows the cyst morphological differences between the cyst nematode species; *H. avenae*, *H. filipjevi* and *H. latipons*.

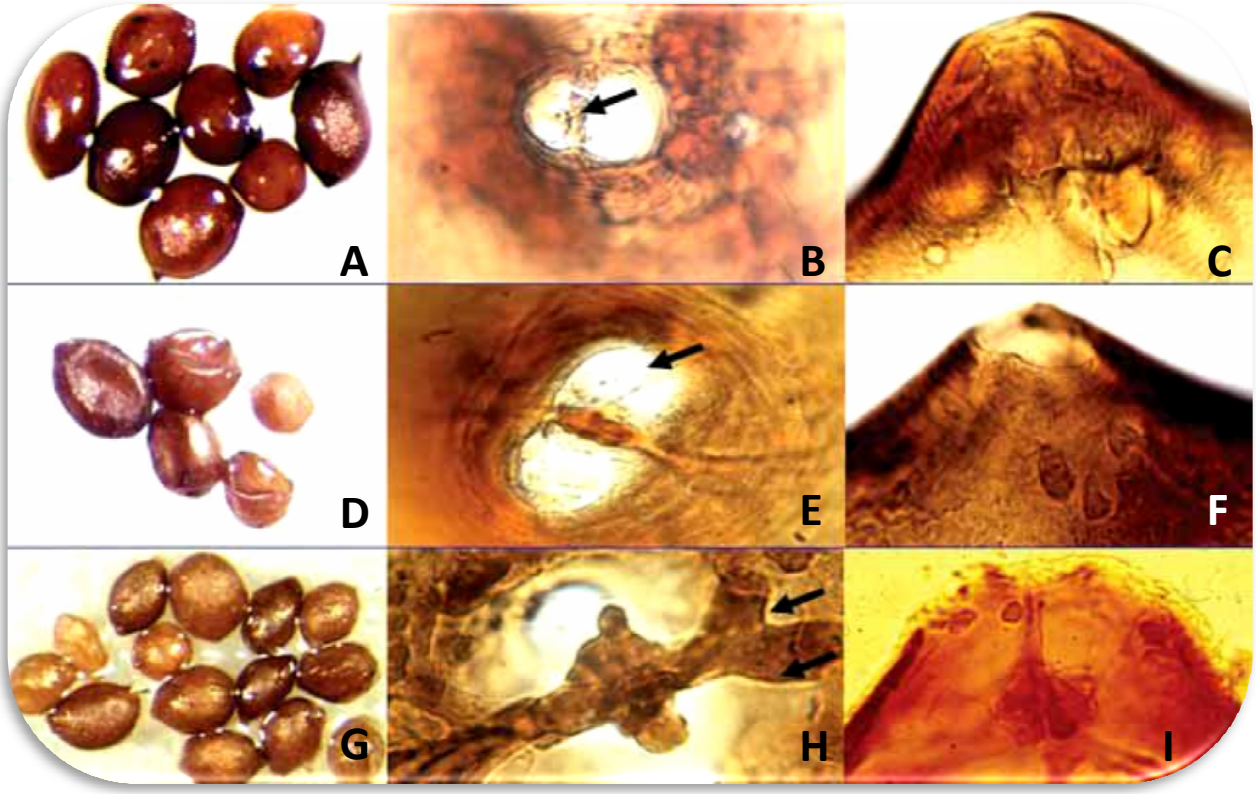

Figure 5. (A-C) Cysts, underbridge and vulval cone of *H. avenae*; **(D-F)** Cysts, underbridge and vulval cone of *H. filipjevi*; **(G-I)** Cysts, underbridge and vulval cone of *H. latipons*. **(Riley *et al.,* 2009)**.

Second-stage juvenile **(Figure 6)** are vermiform, with an offset, dome-shaped head and conical tail tapering to a point. The cuticle is regularly annulated with lateral field lines running from near the head to the tail; the three or four incisures may be reduced in number anteriorly and posteriorly. The head skeleton, stylet and pharynx are well developed, the latter occupying approximately one-third of the body length. The tail hyaline portion is a clear tip of the tail. The tail is approximately 70 µm long, and the hyaline portion is 35–45 µm long. Phasmids are visible in some species as small refractive points lying laterally on the tail surface, usually within the lateral field. Measurements from the J2 are used in diagnosis of genera and species.

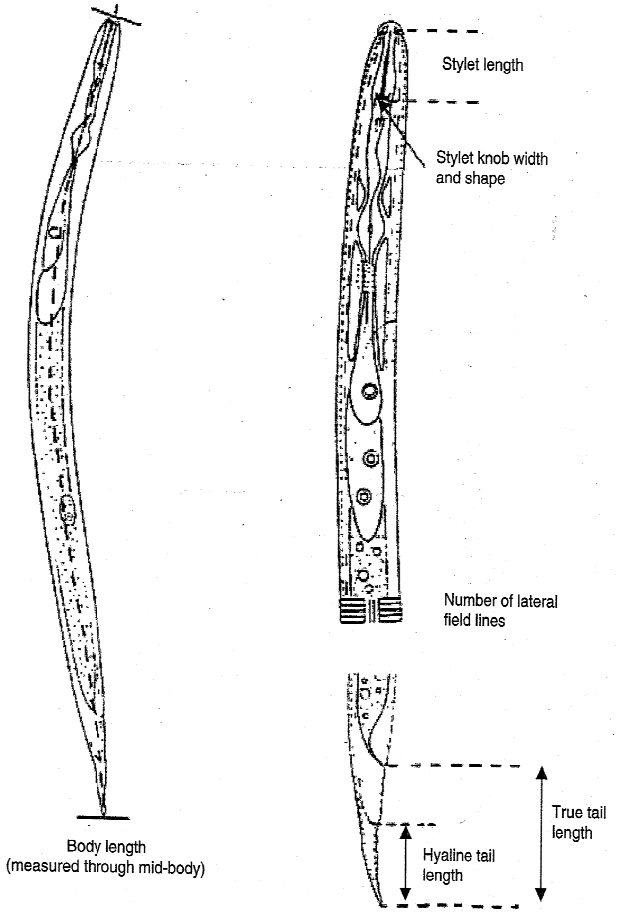

Figure 6. Second-stage juvenile **(Turner and Rowe, 2006).**

Biochemical and molecular techniques based on the analysis of proteins or DNA allow identification of most species of the CCN complex. Molecular markers such as RAPD and PCR-RFLP of rDNA-ITS region have enabled various CCN species to be identified, in addition to exploring the intraspecific variation in several countries as India, China or Saudi Arabia.

Combined morphological and molecular data had defined phylogenetic relationships in the CCN complex and demonstrated two different and separate lineages: the *H. avenae* group containing *H. avenae*, *H. filipjevi* and the *H. latipons* group **(Rivoal *et al.,* 2003, Subbotin *et al.,* 2003, Maafi *et al.,* 2003)**. Until now, however, molecular techniques fail to distinguish pathotypes of CCN **(Subbotin *et al.,* 2002)**.

Symptoms

Plants with heavily damaged roots often appear initially as unthrifty pale green seedlings that occur in patches. Damage may become widespread and uniform over entire fields when susceptible cereals are planted in close crop rotations. Symptoms become more pronounced when affected plants are also exposed to a biotic stresses such as inadequate nutrition, shallow soil, or a shortage of available water. However, nematode-affected plants generally do not respond well to additional applications of fertilizer or water. Plants with heavily damaged roots may be severely stunted and may mature early **(Smiley and Yan, 2010)**.

The symptoms produced on the roots are different dependent on the host. Wheat attacked by *H. avenae* shows increased root production at locations where *H. avenae* females have established a feeding site such that the roots have a 'bushy knotted' **(Figure 7)** appearance usually with several females visible at each knot **(Rivoal and Cook, 1993)**. Nematode invaded roots often fail to grow deeply into the soil. Root tissues invaded by cereal cyst nematodes provide greater opportunities for additional damage by root-rotting fungi and saprophytic bacteria, fungi, and other nematodes **(Smiley and Yan, 2010)**.

Figure 7. Stunted wheat plants with bushy knotted roots heavily infested with *H. avenae* (left) compared non-infested plants (right) **(Riley *et al.,* 2009)**.

Yield losses

Reductions in wheat grain yields by *H. avenae* have been reported from different regions of the world. **Al- Hazmi *et al.* (1999)** reported reduction from 18 to 80% in grain yield of wheat by *H. avenae* in Saudi Arabia. In Morocco, *H. avenae* caused grain yield losses of about 40-50% of wheat **(Rammah, 1994)** and up to 90% of wheat in Spain **(Romero *et al.,* 1988)**. On the Central Anatolian Plateau of Turkey, significant yield losses (average 42%) in several rain-fed winter wheat locations have been reported **(Nicol *et al.,* 2005)**. In Slovakia, *H. avenae* at 180 cysts/kg soil suppressed grain yield by 17-44% for wheat **(Sabova *et al.,* 1981)**. Reductions of wheat yields by *H. avenae* have also been reported from Germany **(Sachse, 1986)**, Libya **(Siddiqui and Khan, 1986)**, France **(Rivoal and Sarr, 1988)**, Italy **(Greco *et al.,* 1993)** and China **(Zhang *et al.,* 1994)**.

In Australia, *H. avenae* decreased the yield of wheat by 20% at a *Pi* of 2 eggs and juveniles/g soil, and 40% at 16 eggs and juveniles/g soil **(Meagher and Brown, 1974).** In Asia, the damage threshold of *H. avenae* in the temperate semi-arid regions of India is considered to be 5-20 eggs and juveniles/g soil for wheat **(Gill and Swarup, 1971; Dhawan and Nagesh, 1987). Mathur *et al.,* (1986)** reported wheat yield losses in India, due to *H. avenae* ranging from 32.4 to 66.5% with nematode densities varying from 4.6 to 10.6 eggs and juveniles/ml soil.

The degree of damage depends on initial population, type of soil, climatic conditions, crop species and cultivar, and interaction with secondary pathogens **(Brown, 1984, 1987)**.

Integrated management

Many examples around the world have shown that the population of CCNs can be reduced effectively through an integrated approach such as:

- Cultural practices

Cultural practices are the most efficient methods of reducing CCNs. One of the most efficient methods of controlling *H. avenae* is with the use of grassfree rotations using non-host crops. In long term experiments, non-host or resistant cereal frequencies of 50% (80% in lighter soils) keep populations below damaging thresholds **(Rivoal and Besse, 1982; Fisher and Hancock, 1991)**. **Mathur (1969)** in India reported that oil cakes, farm yard manure, compost and saw dust applications improved plant growth and subdued multiplication of CCN. Previous studies demonstrated delay in sowing time could escape synchrony between peak emergence of juveniles and the more sensitive early stages of the hosting crop, which permitted to maximize the production of wheat **(Brig Bhan and Kanwar, 2003; Singh and Singh, 2005)**. Under fallow, non-host, or resistant cultivars, populations of *H. avenae* can decline by 70-80% annually through spontaneous hatching which results in the death of juveniles **(Singh *et al.,* 2009)**.

- Chemical control

Nematicides applied to both soil and seeds have provided effective and economical control of CCNs, in Australia, India, and Israel **(Rivoal and Nicol 2009)**. Chemical control is still widely under-used in developing countries. However, the use of chemicals becomes economic when other methods of control are too costly, difficult to apply, or when a method such as rotation is inadequate **(Hague and Gowen 1987)**. Today however, no chemical is considered adequate because of costs, environmental hazards, and high health risks for farmers.

- Biological control

It has been shown that fungal pathogens of nematodes such as *Catenaria auxiliaries* **(Crump *et al.,* 1983; Stirling and Kerry, 1983)**, *Pochonia chlamydosporia* **(Kerry and Crump, 1977)** and *Nematophthora gynophila* **(Kerry and Crump, 1980)** could infect and kill the eggs and females of CCNs. The use of parasites as the nematophagous fungus *Paecilomyces lilacinus*, predators as the trapping fungus *Monacrosporium lysipagum*, and the nematode *Seinura paratenuicaudata* which act on living and mobile stages provided, in laboratory experiments, offer some promise to control *H. avenae* **(Vats, 2004; Khan, *et al.*, 2006)**. Unfortunately the biocontrol treatments by these antagonists have never been commercially feasible. The potential antagonistic microorganisms selected from *in vitro* tests or greenhouse trials often fail to effectively control nematode diseases in field trials. Several factors such as the type and the content of organic matter, pH, nutrient level, and moisture level of the soil influence the efficacy of the biocontrol agents. Due to the variations in environmental factors from one place to other places, sometimes, a good biocontrol agent under *in vitro* conditions fails in *in vivo* conditions. To achieve the success, the environmental factors should be similar to those from which the biocontrol agents were isolated. Likewise, the method of application can influence the success of field trials.

- Host plant resistance

The use of resistant cultivars which leads to a reduction of nematode populations offers one of the most effective control methods with minimum cost or equipment **(Rivoal and Nicol 2009)**. Resistance is defined as the ability of a variety to greatly suppress or prevent reproduction of the nematode. In contrast, when the nematode is capable of multiplying the variety is considered susceptible. The response of a cultivar may range from resistant to susceptible depending on the pest population. Because nematode reproduction is the critical measure for resistance tests, most reports of resistance are based upon research under controlled conditions in the greenhouse. These tests require that plants are placed in soil with a known number of nematodes at the beginning of the test and then grown to the flowering stage **(Smiley *et al.,* 2013)**.

The number of nematodes is determined at the end of the test to determine a reproductive index. It is also possible to estimate resistance by collecting soil samples to measure the initial and final densities of CCN in field plots **(Rivoal and Nicol, 2009)**.

The use of host resistance is an effective method of controlling cereal cyst nematodes. The benefit of resistance is that it reduces the risk for the next susceptible crop. However, even when reproduction is prevented or suppressed, infective juveniles usually invade and injure roots of resistant plants, which can reduce yield **(Smiley *et al.,* 2013)**. Ideally resistance should be combined with tolerance. Tolerance is defined as the ability of a plant to withstand or recover from nematode invasion and to yield well in comparison with non-invaded plants in the same field or region **(Trudgill, 1991).** In contrast, when yield is reduced substantially by the nematode the variety is characterized as being intolerant (or sensitive). The response of an individual variety may range from tolerant to intolerant. Grain yield is used to define tolerance in wheat. Therefore, essentially all reports of tolerance are based upon research in fields that are naturally-infested by the nematode **(Trudgill, 1991)**. Resistance and tolerance are genetically independent, and both are required to reduce the level of risk to future plantings (e.g., resistance) as well as for optimal performance in existing plantings (e.g., tolerance) **(Smiley *et al.,* 2013)**.

Cultivar resistance is considered one of the best methods for nematode control and has been found to be successful in several countries such as Australia, Sweden and France **(Rivoal and Nicol, 2009)**. However, it has also been observed that the use of resistance may lead to replacement of one species with other nematode species, such as *Pratylenchus* **(Lasserre *et al.,* 1994)**. Another concern is the breakdown of resistance sources. This has occurred in France with the resistant oat cultivar Panema and the appearance of a new *H. avenae* pathotype **(Lasserre *et al.,* 1996)**.

In order for cultivar resistance to be effective and durable, knowledge on populations struture (species and pathotypes) is essential. The virulence of populations can be determined by their ability to overcome resistance genes. Such virulent populations may be classified as pathotypes and differentiation of pathotypes can be

carried out using an International Test Assortment of barley, oat and wheat cultivars with respective resistance genes **(Andersen and Andersen (1982b); Persondedryver and Doussinault (1984); Sanchez and Zancada (1987); Rivoal and Cook (1993); Kretschmer *et al.,* (1997); Cook and Rivoal (1998); Cook and Noel (2002); Nicol (2002); Smiley *et al.,* (2011))**.

This test distinguishes three groups of pathotypes of *H. avenae* by differential resistance or susceptibility reactions **(Nicol and Rivoal 2007)**. Groups 1 and 2 include the largest number of pathotypes that occur in Europe, North Africa, and Asia **(Al-Hazmi *et al.,* 2001; Andersen and Andersen 1982a; Mokabli *et al.,* 2002)**. Pathotypes within Group 3 have been identified mostly in Australia and Europe **(Nicol and Rivoal 2007)**. Characterization of the pathotype(s) of *H. avenae* at each location is required to develop and employ resistant cultivars **(Al Hazmi *et al.,* 2001)** that can be used as a component of integrated nematode management systems.

Thesis objectives

The objectives of the present study were:

1- To study the occurrence and to characterize cereal cyst nematodes present in Egypt based on morphometrics, RFLP, and rDNA-ITS sequence analyses.

2- To investigate the influence of different temperature and storage conditions on the hatch of cereal cyst nematode (*Heterodera avenae*) populations from Egypt.

3- To characterize the virulence of cereal cyst nematode (*Heterodera avenae*) populations present in Egypt.

4- To assess the host status of different wheat cultivars to Egyptian populations of the cereal cyst nematode *Heterodera avenae.*

5- To study the influence of initial population densities of cereal cyst nematode (*Heterodera avenae*) on the nematode reproduction on different wheat cultivars.

6- To investigate the influence of initial population densities of cereal cyst nematode (*Heterodera avenae*) on damage potential on different wheat cultivars.

Thesis outline

Chapter 1: Gives an overview about the importance and production of wheat worldwide and in Egypt. Presents general information about cereal cyst nematodes including: distribution; biology and life cycle; hatching and temperature requirements; identification and characterization; symptoms; yield losses; management and integrated control. The chapter gives information on using host resistance as an effective method of controlling cereal cyst nematodes.

Chapter 2: Provides information on the occurrence and distribution of cereal cyst nematode in wheat fields in Ismailia and West Sinai of Egypt. This chapter presents morphological and morphometrical analysis of cysts and second stage juveniles of some Egyptian populations in comparison to cyst-forming nematode populations from Germany. The molecular characteristics of some Egyptian populations based on RFLP and rDNA-ITS sequence analyses were described and compared to some German populations.

Chapter 3: Characterizes the temperature requirements for hatching of five Egyptian populations of the cereal cyst nematode *Heterodera avenae*. Hatching was studied at different temperatures as well as varying temperature regimes simulating seasonal variations in the two different climatic areas. The information in this chapter is valuable for the production and availability of juveniles. This facilitates the investigations on cereal cyst nematode as work is often restricted by lack of juveniles.

Chapter 4: Characterizes the virulence of some cereal cyst nematode populations (*Heterodera avenae*) from Egypt by testing against a number of discriminating wheat cultivars. This chapter describes resistance and tolerance of some local Egyptian wheat cultivars to cereal cyst nematode populations. The generated information may be useful in breeding programs to generate locally adapted resistant or tolerant wheat germplasm in Egypt.

Chapter 5: Determines the influence of initial nematode population density of the cereal cyst nematode (*Heterodera avenae*) on the yield as well as on other plant growth parameters of different wheat cultivars. Assesses the influence of initial nematode population densities of the cereal cyst nematode *Heterodera avenae* on the reproduction potential on different wheat cultivars. This information will be valuable in designing field trials for the development of management strategies to limit damage caused by the nematode in Egypt.

Chapter 6: Summarizes and discusses the overall studies, highlights the main findings in the Ph.D. thesis.

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CHAPTER 2

Occurrence and characterization of cereal cyst nematode in Egypt based on morphometrics, RFLP and rDNA-ITS sequence analyses

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ABSTRACT

A survey on cereal cyst nematodes (CCN) was carried out in wheat production areas in Ismailia province, Egypt. CCN were found in five out of seven regions in Ismailia. The highest incidence of CCN was found in El Shark (West Sinai). Morphological and molecular diversity among the surveyed populations were investigated using light microscopy, ITS-RFLP and sequencing of the rDNA-ITS. The Egyptian populations were identified as *H. avenae* according to morphometrics of cyst vulval cone and second stage juveniles. No differences in ITS-RFLP patterns generated by seventeen restriction enzymes were detected among the Egyptian populations; however the Egyptian populations could be distinguished from German populations of *H. avenae* and *H. filipjevi*. The analyses of ITS region sequences confirmed the species identification of the Egyptian populations, as they were clustered with *H. avenae* populations from Iran, Saudi Arabia, India, Israel and China. This study confirmed the presence of *H. avenae* type B in Ismailia province and West Sinai, as well highlighted its morphological and molecular characteristics. Moreover, the genetic variation presented in this study between the Egyptian and the German populations of *H. avenae* may improve detection and identification among different geographical populations of CCN.

Keywords - Egypt, wheat, cereal cyst nematode, *Heterodera avenae*, occurrence, morphometrics, molecular, RFLP, rDNA-ITS sequences

INTRODUCTION

Cereal cyst nematodes (CCN) are a group of several closely related species which have been documented as causing economic yield loss in wheat production systems in several parts of the world including North Africa, West Asia, China, India, Australia, the United States of America and countries in Europe **(Nicol and Rivoal 2008)**. Three species (*Heterodera avenae, H. filipjevi, and H. latipons*) are among the most economically important cyst nematode pests to cultivated cereals **(McDonald and Nicol 2005; Rivoal and Cook 1993)**. *H. avenae* is widely distributed in temperate wheat-producing regions throughout the world **(Nicol *et al.,* 2003; Nicol 2002; Rivoal and Cook 1993; Smiley and Nicol 2009)**. *H. filipjevi* is found in eastern and northern Europe, central and west Asia, the Middle East, the Indian subcontinent, and North America **(Rivoal *et al.,* 2003; Smiley and Nicol 2009; Smiley *et al.,* 2005)**. *H. latipons* occurs mainly in the Mediterranean region but also in Asia and Europe **(Abidou *et al.,* 2005; Smiley and Nicol 2009)**.

The first record of a species from *H. avenae* group on wheat in Egypt was by **Ibrahim *et al.,* in 1986**, they found *H.avenae* on barley and wheat in the Nile Delta (EL-Behera governorate). Furthermore, **Ibrahim and Handoo** in **2007** reported the occurrence of *H.avenae* on Egyptian wheat in a survey conducted in Alexandria and El-Behera Governorates in northern Egypt. Despite of these reports, still little is known about the occurrence and distribution of cereal cyst nematode on wheat in Egypt. Consequently, more information about the distribution of CCN in the Egyptian wheat belt, adds importance to the question of dissemination and its potential for future spread.

The taxonomy of the *H. avenae* group has been advanced by numerous review papers published by different workers **(Mulvey and Golden 1983; Ferris *et al.,* 1989, 1994; Golden, 1986; Krall, 1977; Mulvey, 1972, 1973; Robinson *et al.,* 1996; Rumpenhorst *et al.,* 1996; Stone and Hill, 1982; Vovlas, 1985; Williams and Siddiqi, 1972; Wouts and Sturhan, 1995; Wouts *et al.,* 1995)**. This group of nematodes forms a complex of species referred to as the "*H. avenae*-group or complex".

With an increasing number of species in this group, reliable identification based on morphology is becoming more difficult **(Subbotin *et al.,* 2003)**. Knowledge on the genetic variability present among different geographical populations is becoming essential for the development of suitable management strategies. Internal transcribed spacer regions of ribosomal genes (rDNA-ITS) were found to be useful to differentiate species within the *H. avenae* group **(Ferris *et al.,* 1994; Bekal *et al.,* 1997)**.

The aim of this study was to provide information on the occurrence and distribution of CCN species in wheat fields in Ismailia and West Sinai of Egypt. This study presents a morphological and morphometrics analysis of cysts and second stage juveniles of some Egyptian populations compared to cyst-forming nematode populations from Germany. The molecular characteristics of some Egyptian populations based on RFLP and rDNA-ITS sequence analyses were described and compared to some German populations to enhance the knowledge about the molecular variation of different geographical populations of *H. avenae* group members.

MATERIALS AND METHODS

Sampling, Extraction and Estimation

1- Sampling Procedures: -

The survey study was carried out during the wheat growing season (2008-2009) in seven different localities representing the main wheat growing areas in Ismailia province and West Sinai. The sites were located at Abu Khalifah, Abu Suwayr, El Kasasen, El Shark (West Sinai), El Wasfia, Serabeum regions, and the Experimental Farm of the Faculty of Agriculture, Suez Canal University **(Figure 1)**. Sampling was done by divided the fields systematically by a 10 x 10 m network of gridlines. Soil subsamples were collected at every 3-4 m in a 'W' pattern. Soil sample of about 1 kg was composed of 10 randomized subsamples obtained from each grid intersections at depth of 10-25 cm. A total of 315 composite soil and root samples were collected from the rhizosphere of wheat plants. Soil samples were placed in a plastic bag, labeled with its grid number and kept in refrigerator at 5˚C for further studies.

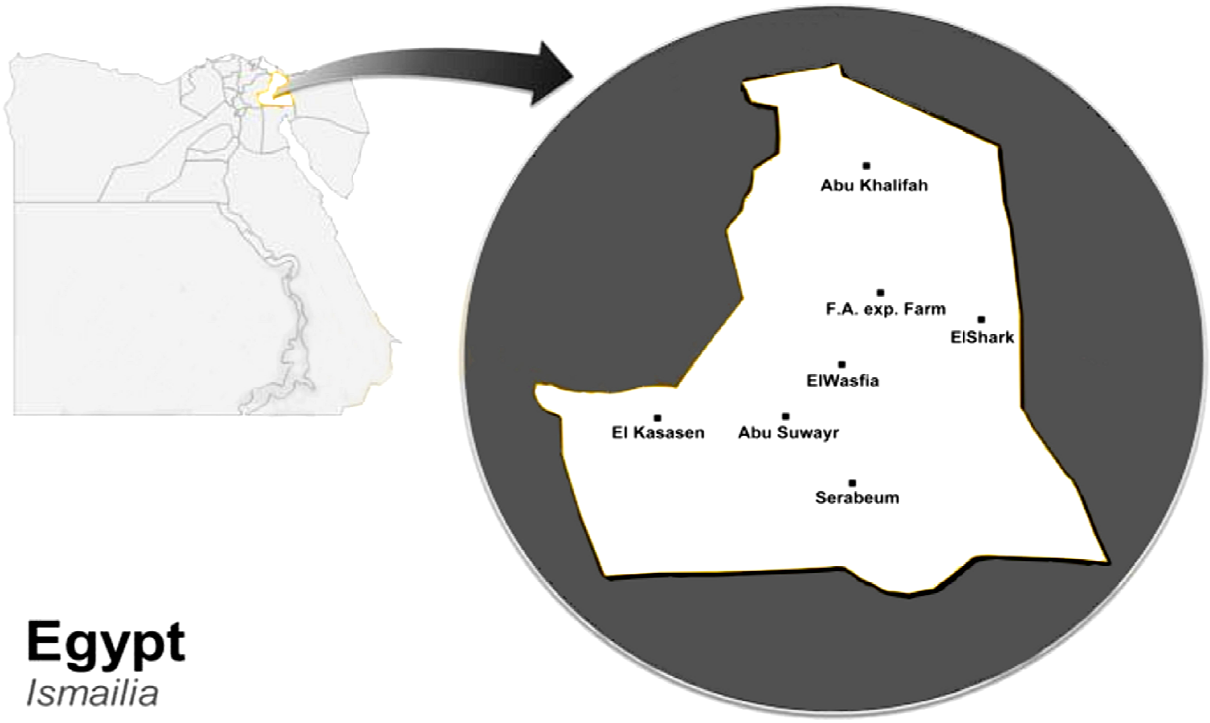

Figure 1. The distribution of different locations sampled for cereal cyst nematode in Ismailia province.

2- Nematode Extraction and Counting:-

Each soil sample was carefully mixed and a volume of 100 cm^3 soil was used to extract second-stage juveniles (J2) using sieving and Baermann dish technique according to **Barker *et al.,* (1985)**. The extracted juveniles were counted by using 1 ml Hawksley counting slide under compound microscope (Axiovert 25) at 100x magnification. A volume of 100 cm^3 soil was used to extract cysts using floatation technique **(Shepherd, 1986)**. Counting and separation of cysts from soil debris and other organic materials retained on the filter paper were carried out under a stereoscopic binocular at 25x magnification (Leica MZ8). Roots were carefully washed free of adhering soil and the white females were removed and counted under a stereoscopic binocular (Leica MZ8) at 25x magnification.

Morphology and Morphometric studies

Descriptions and measurements of eggs, cysts and J2s of the surveyed populations compared to three different cyst nematode populations from Germany; *H. avenae* (Grafenreuth population), *H. filipjevi* (Rädel population) and *H. schachtii* (Münster population) were carried out (**Table 1**). Cone tops (posterior ends of cysts) were prepared as described by **Mulvey, 1972**. J2s were obtained after squashing cysts soaked in water for 5 days then temporary mounts were made in water on glass slides and immediately examined. Six morphometrical characters of the cone tops (semifenestral length, semifenestral width, vulval slit length, vulval bridge length, vulval bridge width and under bridge length) along with thirteen morphometrical characters of the J2s (body length (L), midbody width(W), L/W ratio, head height (HH), head width (HW), HH/HW ratio, stylet length (SL), stylet knobs width (SW), tail length (TL), tail width at anus (TW), tail hyaline region (HR), HR/SL ration and tail lateral lines) were measured under a compound microscope (Axiovert 25) using a calibrated ocular micrometer. Species identification was based on cysts vulval cone structures and measurement, as well as, morphometric features of J2s according to **Mulvey and Golden, 1983; Lamberti and Taylor, 1986; Sharma and Sharma, 1998**.

Table 1. Origin of nematode populations of the genus *Heterodera* used in this study.

Population code	Species	Source
AK	*Heterodera spp.*	Abu Khalifah region, Ismailia, Egypt
AS	*Heterodera spp.*	Abu Suwayr region, Ismailia, Egypt
EK	*Heterodera spp.*	El Kasasen region, Ismailia, Egypt
ES	*Heterodera spp.*	El Shark region (West Sinai), Ismailia, Egypt
SB	*Heterodera spp.*	Serabeum region, Ismailia, Egypt
HA [a]	*Heterodera avenae*	Grafenreuth, Germany
HF [a]	*Heterodera filipjevi*	Rädel, Germany
HS [a]	*Heterodera schachtii*	Münster, Germany

[a] These populations were obtained from Julius Kühn-Institut, Germany.

Principal component analysis (PCA) was performed on six morphometrical characters of the cysts cone tops as well as on thirteen morphometrical characters of the J2s to establish the relationships among the tested populations. The detection of factors present in the original data matrix was determined by PCA through the calculation of new variables (principal components) that summarize the information dispersed in the original variables. The eigenvalues produced by PCA are the contribution of each component to the original variance and their correlation coefficients with the original variables. Scatter plots of two dimensional correlation based principal component analysis of cone tops and J2s morphometrical data of the tested populations were performed.

Data Analysis

For each surveyed site, population density (P.D.) of nematode per 100 cm^3 soil or plant root system, frequency of occurrence % (F.O.%) and prominence value (P.V.) were calculated according to **(Norton, 1978)** as follows: P.D. = average number of nematodes per 100 cm^3 soil or plant root system. F.O.% = (number of samples containing a nematode / total number of collected samples)*100. P.V. = population density $\sqrt{}$ frequency of occurrence. Levene's test was used to test the homogeneity of variance. All data were analyzed using ANOVA (SPSS version 19.0, IBM Corporation, New Orchard Road Armonk, New York, United States). Means and standard deviations of the mean were separated using Tukey HSD test at $P \leq 0.05$.

DNA extraction and PCR reaction

DNA was extracted from cysts of eight *Heterodera* populations **(Table 1**) using DNeasy Tissue Kit (Qiagen, Hilden, Germany). A pair of 21-mer primers localized at the extremities of the 18S and 26S ribosomal genes originally isolated by **(Vrain *et al.,* 1992)** and synthesized by Invitrogen GMBH (Darmstadt, Germany) were used in the PCR reaction. The forward primer 18S (5'-TTG-ATT-ACG-TCC-CTG-CCC-TTT-3') and the reverse primer 26S (5'-TTT-CAC-TCG-CCG-TTA-CTA-AGG-3'), were used to amplify the two internal transcribed regions, ITS1 and ITS2, and the 5.8S gene of *Heterodera* populations.

Polymerase Chain Reactions (PCR) were carried out in 50 µl reaction volumes, containing 5 µl 10x PCR buffer, 3 µl 25 mM $MgCl_2$, 3µl 10 mM each primer, 3 µl 2 mM each of dATP, dCTP, dGTP, and dTTP, 0.8 µl Taq DNA polymerase 1 U/µl (Fermentas life science GMBH, St. Leon-Rot, Germany), 27.2 µl distilled water and 5 µl template DNA. The PCR amplification profile carried out in an Eppendorf Thermal cycler ('Mastercycler® 5333', Eppendorf AG, Hamburg, Germany). DNA thermal cycler was programmed for 1 cycle of 5 min at 94 °C; and 40 cycles of 94 °C for 1 min, 60 °C for 1 min and 72 °C for 2 min; followed by a final elongation step of 7 min at 72 °C. A negative control containing the PCR mixture without any DNA template was also run.

After PCR amplification, 5 µl of each PCR product was mixed with 1 µl of 6x loading buffer (Fermentas life science GMBH, St. Leon-Rot, Germany) and loaded on a 1 % standard TBE buffered agarose gel. Five µl of DNA ladder 100bp 0.5 µg/µl (Fermentas life science GMBH, St. Leon-Rot, Germany), was loaded on the first line of the gel. After electrophoresis (100 V for 40 min) the gel was stained with ethidium bromide (0.1 µg/ml) for 20 min, visualized and photographed under UV-light. The remaining PCR products were stored at –20 °C.

PCR and RFLP of the ITS-rDNA

Ten µl of each PCR-product of *Heterodera* populations was digested with one of the following seventeen restriction enzymes: *AluI, AvaI, Bsh1236I, DraI, HaeIII, HhaI, HinfI, Hinp1 I, Hypyf3I (DdeI), MspI, MvaI, PstI, PvuII, RsaI, Sau3AI, TaqI* and *Tru1I* (Fermentas life science GMBH, St. Leon-Rot, Germany), in the buffer stipulated by the manufacturer. Procedures for obtaining PCR amplified products and endonuclease digestions of these products were repeated several times to verify the results. Digested DNA was loaded on a 1.5% agarose gel, separated by electrophoresis, stained with ethidium bromide, visualized under UV transilluminator, photographed by Intas Gel imager system (Intas science imaging instruments GMBH, Göttingen, Germany) and analyzed using Gel compare II v6.5 software (Applied Maths NV, Sint-Martens-Latem, Belgium). The relationships among species and populations were analyzed using coefficient of similarity (JACCARD) based on bands molecular sizes. Cluster analysis with the unweighted pair-group method using arithmetic averages (UPGMA) was applied to compile a dendrogram clustering the populations at different levels on a scale of similarity. Bootstrap analysis using 1000 bootstrapped data sets, was performed to determine statistical consistency of the classification.

Sequencing and Phylogenetic Analyses

PCR products of *Heterodera* populations were purified using High Pure PCR Product Purification Kit (Roche Diagnostics GmbH, Mannheim, Germany). DNA from each sample was sequenced (IIT Biotech, Biologie/Zentrales Isotopenlabor, Bielefeld University, Bielefeld, Germany) in both directions to obtain overlapping sequences of both DNA strand. The sequences were edited and analyzed using software packages of Chromas 2.01 (Digital River GmbH, Shannon Free Zone West, Shannon Co. Clare, Ireland) and MEGA 5.05 (Center for Evolutionary Medicine and Informatics, The Biodesign Institute, S. McAllister Ave, Tempe, AZ, USA). All sequences were blasted in GenBank (http://www.ncbi.nlm.nih.gov/) to reveal its origin (*Heterodera* species and DNA-region). Sequence alignment of 29 sequences (8 new and 21 known ITS sequences from GenBank database, **Table 2**) was analyzed using the Neighbor-Joining method by

Geneious® Pro 5.6.6 software (Biomatters Ltd, 76 Anzac Avenue, Auckland 1010, New Zealand, http://www.geneious.com). A consensus tree clustering the populations at different levels based on genetic distance (Tamura-Nei model) was constructed from the ITS sequence alignment. To determine statistical consistency of the classification, bootstrap analysis using 1000 bootstrapped data sets, was performed. (The new sequences in this study were deposited in GenBank under accession numbers; KF225719, KF225720, KF225721, KF225722, KF225723, KF225724, KF225725 and KF225726; indicated in **Table 2**).

Table 2. The nematode species and populations used in sequencing and phylogenetic analyses.

Species	Accession number	Population	Country	Source of the material or data
H. avenae	KF225719*	Abu Khalifah **(AK)**	Egypt	Survey in Abu Khalifah region
	KF225720*	Abu Suwayr **(AS)**	Egypt	Survey in Abu Suwayr region
	KF225721*	El Kasasen **(EK)**	Egypt	Survey in El Kasasen region
	KF225722*	El Shark **(ES)**	Egypt	Survey in El Shark region (West Sinai)
	KF225723*	Serabeum **(SB)**	Egypt	Survey in Serabeum region
	HM560754	Juqiao village, Henan	China	Peng *et al.,* Unpublished
	AY148359	Nuisement sur Coole	France	Subbotin *et al* 2003
	KF225724*	Grafenreuth **(HA)**	Germany	Julius Kühn-Institut
	AY148360	Dedesdorf	Germany	Subbotin *et al.,* 2003
	AY148362	near Delhi	India	Subbotin *et al.,* 2003
	AF498378	Ilam, Mehran-Reza Abad	Iran	Tanha Maafi *et al.,* 2003
	AY148363	Bet Dagan	Israel	Subbotin *et al.,* 2003
	AY148361	Unknown	Saudi Arabia	Subbotin *et al.,* 2003
	AY148356	Santa Olalla	Spain	Subbotin *et al.,* 2003
	AY148358	IACR-Rothamsted	UK	Subbotin *et al.,* 2003
H.filipjevi	GU083596	Xuchang, Henan province	China	Peng *et al.,* 2010
	KF225725*	Rädel **(HF)**	Germany	Julius Kühn-Institut
	AY148400	Gimbte	Germany	Subbotin *et al* 2003
	GU565575	Iran 4	Iran	Mokaram *et al.,* Unpublished
	AY347922	Foggia	Italy	Madani *et al.,* 2004
	AY148399	Torralba de Calatrava	Spain	Subbotin *et al.,* 2003
	AY148398	Selcuklu	Turkey	Subbotin *et al.,* 2003
	GU079654	Unknown	USA	Yan and Smiley 2010
H.schachtii	EF611100	Momalle	Belgium	Madani *et al.,* 2007
	EF611103	Aisne	France	Madani *et al.,* 2007
	KF225726*	Münster **(HS)**	Germany	Julius Kühn-Institut
	EF611115	Schladen	Germany	Madani *et al.,* 2007
	EF611102	Borsel	Netherland	Madani *et al.,* 2007
	JX024219	Unknown	Poland	Toumi *et al.,* Unpublished

* New sequences

RESULTS

Occurrence of cereal cyst nematode

Cereal cyst nematodes (CCN) were found in five out of seven regions in Ismailia governorate, with a frequency of occurrence of 79.4% in a total of 315 collected composite soil samples **(Table 3)**. CCN population density was on average 29 cysts and 10 juveniles per 100 cm^3 soil and on average 2 females per plant were found. No CCN were detected in El Wasfia region and in the Experimental Farm of the Faculty of Agriculture after examining 31 and 34 composite soil and root samples, respectively. In El Shark location (West Sinai), the highest incidence of CCN was detected with population density of 45.6 cysts/100 cm^3 soil and an abundance of occurrence 92%. CNN were detected in Serabeum region with densities 29.4 cysts/100 cm^3 soil and frequency of occurrence of 87%. On the other hand, the lowest abundance and incidence of CCN were found in samples from Abu Suwayr and Abu Khalifah regions. The incidence of CCN in Abu Khalifah was 14.5 cysts/100 cm^3 soil, while the frequency of occurrence was 83%. Abu Suwayr region showed average number of cysts 13.9/100 cm^3 soil, and abundance of occurrence of 79%.

Table 3. Occurrence of cereal cyst nematode in wheat fields of Ismailia governorate.

Regions		Abu Khalifah	Abu Suwayr	El Kasasen	El Shark (West Sinai)	Serabeum
No. of samples		39	57	42	64	48
Cysts/100 cm^3 Soil	*P.D.±S.D.*	14.5±10.9 **c**	13.9±9.5 **c**	33.6±20 **b**	45.6±17.5 **a**	29.4±12.3 **b**
	F.O.%	83	79	89	92	87
	P.V.	13.2	12.4	31.7	43.7	27.5
Juveniles/100 cm^3 Soil	*P.D.±S.D.*	7.9±5.1 **cd**	6.0±4.8 **d**	12.2±5.8 **ab**	13.5±6.5 **a**	9.6±6.4 **bc**
	F.O.%	79	70	85	91	78
	P.V.	7.1	5.0	11.2	12.9	8.5
Females/ plant root	*P.D.±S.D.*	1.3±1.1 **bc**	1.0±0.9 **c**	1.6±1.0 **ab**	1.9±0.7 **a**	1.6±1.0 **ab**
	F.O.%	65	55	74	81	75
	P.V.	1.1	0.7	1.4	1.7	1.4

P.D. (Population Density) = Total number of nematodes per 100 cm^3 soil or plant root system
F.O.% (Frequency of Occurrence %) = (Number of samples contained a nematode / Total samples collected)*100
P.V. (Prominence Value) = Population density √ Frequency of Occurrence
Means in the same raw followed by the same litter(s) were not significantly different according to Tukey HSD test (P≤0.05).

Morphology and Morphometric studies

Morphological identification based on the descriptions and measurements of cysts, eggs, and J2 of the Egyptian populations were carried out. No variations in cyst vulval cone and J2 shape and measurements were noticed among the investigated populations from Egypt. Eggs of all populations were typically ellipsoidal and shell hyaline without any workings. Cysts were variable in size (0.71 mm * 0.50 mm) but mostly lemon-shaped, with protruding neck and vulva. Cysts wall was dark brown to black in color, bearing a zig-zag pattern. New cysts of all populations were mostly enveloped with a subcrystalline layer. Measurements of vulval cone areas of the Egyptian populations were recorded in **Table 4**.

Table 4. Measurements of vulval cone areas of the studied cyst nematode populations (Measurements in µm, n=15, **mean ± standard deviation**, *range*).

population	Egyptian Populations					German Populations		
Characters	Abu Khalifah	Abu Sawyer	El Kasasen	El Shark (West Sinai)	Serabeum	*H. avenae* (Grafenreuth)	*H. filipjevi* (Rädel)	*H. schachtii* (Münster)
Fenestration	Bi-fenstrate	Bi-fenstrate	Bi-fenstrate	Bi-fenstrate	Bi-fenstrate	Bi-fenstrate	Bi-fenstrate	Ambi-fenstrate
Semifenestrae Length	**51.1 ± 3.2 a** *43.9-54.7*	**51.5 ± 2.3 a** *49.0-53.9*	**52.8 ± 3.3 ab** *43.9-54.8*	**52.3 ± 2 ab** *50.9-55.8*	**51.8 ± 2.8 ab** *47.5-54.6*	**50.8 ± 5.0 a** *41.2-53.9*	**56.9 ± 3.6 b** *53.9-63.7*	**68.6 ± 10.1 c** *53.9-83.3*
Semifenestrae Width	**21.8 ± 2.7 a** *18.5-24.5*	**22.3 ± 2.3 ab** *18.5-24.5*	**23.5 ± 1.4 ab** *19.6-24.5*	**22.9 ± 2.1 ab** *19.6-25.5*	**21.8 ± 2.4 ab** *17.6-24.5*	**21.7 ± 2.7 a** *19.6-25.5*	**25 ± 1.8 b** *23.5-29.4*	**32.1 ± 5.1 c** *24.5-39.2*
Vulval slit length	**10.8 ± 1.3 a** *8.8-12.7*	**10.7 ± 0.8 a** *9.8-11.8*	**10.3 ± 1.3 a** *8.8-12.7*	**10.9 ± 1.5 a** *8.9-12.8*	**10.8 ± 1.5 a** *8.9-12.8*	**10.7 ± 1.1 a** *9.8-12.8*	**8.8 ± 1.5 a** *6.9-10.8*	**43.1 ± 5 b** *39.2-49*
Vulval Bridge Length	**33.3 ± 4.4 a** *24.3-38.8*	**32.4 ± 4.3 a** *27.7-39.4*	**34.8 ± 3.8 a** *24.3-40.2*	**36.5 ± 3.6 a** *29.4-41.4*	**35.7 ± 5.2 a** *28.4-40.4*	**32.7 ± 4.9 a** *24.5-39.2*	**36.7 ± 5.1 a** *29.6-44.4*	**57.8 ± 3.8 b** *53.9-63.7*
Vulval Bridge Width	**9.7 ±1.8 a** *7.9-12.7*	**9.5 ± 1.1 a** *7.9-10.8*	**9.8 ± 1.6 a** *6.9-12.7*	**10.2 ± 0.5 a** *9.8-10.8*	**9.9 ± 1.7 a** *6.9-11.8*	**9.4 ± 2 a** *4.9-10.8*	**10.4 ± 0.5 a** *8.9-14.8*	**12.6 ± 2.1 b** *9.8-10.8*
Under Bridge	Absent	Absent	Absent	Absent	Absent	Absent	Medium	Medium
Under Bridge Length	-- --	-- --	-- --	-- --	-- --	-- --	**79.6 ± 7.2 a** *69.4-89*	**94.7 ± 18.3 b** *73.5-127.4*

Means in the same raw followed by the same litter(s) were not significantly different according to Tukey HSD test (P≤0.05).

Fenestration was bi-fenestrate with semifenetrae length ranged between 43.9 - 55.8 µm and width ranged between 17.6 - 25.5 µm. Semifenstra separated by fairly wide vulval bridge with width ranged between 6.9 - 12.7 µm and length ranged from

24.3 to 41.4 µm. Vulval slit short with length ranged between 8.8 - 12.8µm. Underbridge absent and heavy bullae crowded into vulval cone were observed.

Morphometrical data of J2s of the surveyed CCN populations were presented in **Table 5**. Second stage juveniles body typically vermiform, elongate, tapering posteriorly with length ranged between 480 - 624 µm and midbody width ranged between 18.6 - 24.6 µm. Head slightly set off, slightly rounded with four postlabial annules and height ranged between 3.6 - 5.9 µm and width between 8.8 - 11.1 µm. Stylet strong slender with length ranged from 20.6 to 27.5 µm and shallowly concave anteriorly knobs with width ranged between 3.9-5.9 µm. Tail length ranged between 53.80 - 78.40 µm with rounded terminus and width at anus ranged from 14.7 to 18.6 µm. In addition, the hyaline tail-terminal length ranged between 39.2 - 53.9 µm. Tail lateral lines varied from three to four lines but tended to be four in most of the examined specimens. Moreover, large lens-like phasmids were detected.

Variations in characteristics and measurements of cyst vulval cone and second stage juveniles were observed between the Egyptian and the German CCN populations. These variations ranged from slight variation when comparing the Egyptian populations with *H. avenae* (Grafenreuth) to significant variation when comparing the Egyptian populations with *H. filipjevi* (Rädel). Fenestration of Grafenreuth population was similar to the Egyptian populations **(Table 4)**, bi-fenestrate with relative semifenetrae length ranged between 41.2 - 53.9 µm. Vulval bridge length also were very close to the Egyptian populations and ranged between 24.5 - 39.2 µm. In addition, short vulval slit, underbridge absent and heavy bullae were observed into the vulval cone.

Larvae body length and width was in the same range of the Egyptian populations with 480 - 576µm and 20.5 - 23.5 µm, respectively **(Table 5)**. Moreover, head offset with four postlabial annules and height ranged between 3.9 - 5.9 µm. The stylet was strong with length close to the surveyed populations and ranged between 23.5 - 26.5 µm. Shallowly concave anteriorly knobs with width ranged from 4.9 to 5.9 µm were observed. Tail length, tail width at anus and hyaline tail-terminal length seems to close to Egyptian population's measurements and ranged between 58.8 - 70.6 µm, 15.7 - 17.6

µm and 39.2 - 46.1 µm, respectively. Furthermore, four tail lateral lines and large lens-like phasmids were detected.

Table 5. Measurements of second stage juveniles of the studied cyst nematode populations (Measurements in µm, **mean ± standard deviation**, *range*).

Populations	Egyptian Populations					German Populations		
Characters	Abu Khalifah	Abu Sawyer	El Kasasen	El Shark (West Sinai)	Serabeum	*H. avenae* (Grafenreuth)	*H. filipjevi* (Rädel)	*H. schachtii* (Münster)
n =	35	40	35	40	33	45	40	40
Body Length (L)	560.9 ±28.2 a *528-600*	552.6 ± 24.3 a *480-576*	556.3 ± 42.6 a *480-600*	562 ± 38.2 a *480-624*	559.8 ± 25.3 a *504-600*	551.8 ± 25.8 a *480-576*	517.1 ± 14 b *490-552*	452.3 ± 28.1 c *408-504*
Midbody Width (W)	22.7 ± 1.0 a *20.6 -24.5*	22.7 ± 1.2 a *20.60-24.50*	23.1 ± 0.6 a *21.5 - 24.5*	22.8 ± 0.9 a *18.6 - 23.5*	22.9 ± 1.2 a *20.6 - 24.6*	22.6 ± 0.8 ab *20.5 - 23.5*	22 ± 1 b *20.6 - 23.5*	20.6 ± 1 c *18.6 - 22.5*
L/W Ratio	24.8 ± 1.5 a *21.6 -27.8*	24.4 ± 1.5 ab *21.6 - 26.7*	24.2 ± 2.2 ab *20.2 - 27.9*	24.7 ± 1.9 ab *20.4 - 29.7*	24.6 ± 1.8 ab *20.5 – 28*	24.5 ± 1.5 ab *20.4 - 28.1*	23.6 ± 1.1 b *21.5 - 25.6*	22 ± 1.7 c *18.9 - 25.8*
Head Height (HH)	5.1 ± 0.5 a *3.9-5.9*	4.8 ± 0.9 a *3.6 - 5.9*	4.9 ± 0.4 a *3.9 - 5.9*	4.8 ± 0.5 a *3.9 - 5.9*	5.1 ± 0.5 a *3.9 - 5.9*	4.8 ± 0.4 a *3.9 - 5.9*	4.8 ± 0.3 a *3.9 - 4.9*	5.1 ± 0.4 a *4.9 - 6.9*
Head Width (HW)	10.1 ± 0.6 a *8.8 -10.8*	10 ± 0.4 a *9.8 - 11.1*	9.9 ± 0.5 a *8.8 - 10.8*	9.8 ± 0.3 a *8.8 -10.8*	10.1 ± 0.4 a *9.8 - 10.8*	9.8 ± 0.5 a *8.8 - 10.8*	10 ± 0.9 a *8.3 - 10.8*	10 ± 0.3 a *9.8 - 10.8*
HH/HW Ratio	0.5 ± 0.1 a *0.4 - 0.6*	0.5 ± 1 a *0.4 - 0.6*	0.5 ± 0.1 a *0.4 - 0.7*	0.5 ± 0.1 a *0.4 - 0.6*	0.5 ± 0.1 a *0.4 - 0.6*	0.5 ± 0.1 a *0.4 - 0.7*	0.5 ± 0.1 a *0.4 - 0.6*	0.5 ± 0.1 a *0.5 - 0.6*
Stylet Length (SL)	25.2 ± 0.8 a *23.5 - 26.5*	25.4 ± 2 a *22.5 - 27.5*	25.4 ± 0.9 a *23.5 - 26.5*	25.3 ± 1 a *21.6 - 26.5*	25 ± 1.2 a *20.6 - 27.4*	25.3 ± 0.9 a *23.5 - 26.5*	25 ± 2.5 a *19.6 - 27.5*	25.1 ± 0.6 a *23.5 - 26.5*
Stylet Knobs Width (SW)	5.4 ± 0.5 a *4.9 - 5.9*	5.2 ± 0.4 a *4.9 - 5.9*	5.5 ± 0.6 a *4.9 - 5.9*	5.5 ± 0.4 a *4.9 - 5.9*	5.3 ± 0.6 a *3.9 - 5.9*	5.3 ± 0.5 a *4.9 - 5.9*	5.5 ± 0.5 a *4.9 - 5.9*	5.2 ± 0.4 a *4.9 - 5.9*
Tail Length (TL)	70.3 ± 4.2 a *63.7 - 78.4*	69.4 ± 5.4 a *53.8 - 78.4*	70.4 ± 3.2 a *67.6 - 78.4*	70.1 ± 3.8 a *58.8 - 74.5*	69.6 ± 2.9 a *65.7 - 73.5*	69.6 ± 2.8 a *58.8 - 70.6*	56.3 ± 3.5 b *49 - 63.7*	48.7 ± 3.9 c *44.1 - 58.8*
Tail Width at anus (TW)	16.8 ± 0.6 a *15.7 - 17.6*	16.8 ± 1 a *14.7 - 17.9*	17.1 ± 0.4 a *16.7 - 17.6*	17 ± 1 a *15.7 - 18.6*	16.6 ± 0.8 a *14.7 - 17.6*	16.5 ± 0.7 ab *15.7 - 17.6*	15.9 ± 1.5 b *13.7 - 17.6*	13.5 ± 1.1 c *10.8 - 15.7*
Tail Hyaline region (HR)	46.1 ± 2.3 a *42.2 - 53.9*	45.6 ± 2 a *43.1 - 49*	45.6 ± 2.1 a *44.1 - 51*	45.7 ± 2.5 a *44.1 - 52.9*	45 ± 2.4 a *39.2 – 49*	44.8 ± 2.2 a *39.2 - 46.1*	37.1 ± 3.1 b *29.4 - 44.1*	29.9 ± 4.1 c *24.5 - 39.2*
HR / SL Ratio	1.8 ± 0.1 a *1.7 - 2.2*	1.8 ± 0.2 a *1.6 -2.2*	1.8 ± 0.1 a *1.7 -1.9*	1.8 ± 0.1 a *1.7 -2.2*	1.8 ± 0.2 a *1.5 - 2.1*	1.8 ±0.1 a *1.6 -2*	1.5 ± 0.2 b *1.2-2*	1.2 ± 0.2 c *1-1.5*
Tail Lateral lines	4	3-4	4	4	4	4	3-4	3-4

Means in the same raw followed by the same litter(s) were not significantly different according to Tukey HSD test (P≤0.05).

Similar to the Egyptian populations, *H. filipjevi* (Rädel) showed bi-fenestrate fenestration but with semifenetrae length and width significantly higher and ranged between 53.9 - 63.7 µm and 23.5 - 29.4 µm, respectively **(Table 4)**. Vulval bridge length and width were close with slight different and ranged between 29.6 - 44.4 µm and 9.8 - 10.8 µm, respectively. Vulval slit was shorter and ranged in length between 6.9 - 10.8

µm, underbridge was present with medium development and length ranged between 69.4 - 89 µm, heavy bullae was observed. Larvae body length of *H. filipjevi* was significantly shorter and ranged between 490 - 552 µm **(Table 5)**. Head was offset from the rest of body similar to the Egyptian populations but just two annules were found. Tail length was remarkably shorter and ranged between 49 - 63.7 µm with rounded terminus and width at anus ranged from 13.7 to 17.6 µm. Moreover, the hyaline tail-terminal was somewhat shorter and ranged in length between 29.4 - 44.1 µm. Three to four tail lateral lines and large lens-like phasmids were detected. On the other hand, *H. schachtii* (Münster population) showed several significant variations from the surveyed populations **(Table 4 and 5)**.

CCN populations found infecting wheat fields in different regions of Ismailia governorate, Egypt were identified as a *Heterodera avenae* mainly by the characteristics and measurements in **Table 4** and **5** which confirmed the identification according to **Mulvey and Golden, 1983; Lamberti and Taylor, 1986; Sharma and Sharma, 1998**.

PCA plots based on morphometrical values of cone tops **(Figure 1-A)** and second stage juveniles **(Figure 1-B)** using KMO and Bartlett's test, showed that the analyzed populations can be significantly ($P < 0.001$) separated into two well separated groups. One group is represented by the Egyptians populations and the German population of *H. avenae* (Grafenreuth), and the second is represented by *H. filipjevi* (Rädel). In both PCA of cone top and J2 characteristics, the first two components accounted for 76.2 and 85.7% of the total variance, respectively. Semifenestral length (eigenvalue 0.80) and under bridge length (eigenvalue 0.84) had the highest correlation with the first principal component, suggesting a high discriminative power **(Figure 1-A)**. Vulval slit length (eigenvalue 0.64) and vulval bridge length (eigenvalue 0.84) was best correlated with the second variable.

Figure 1-B showed the PCA of J2 morphometrical characters of the tested populations, the tail hyaline region (eigenvalue 0.92), tail length (eigenvalue 0.79) and ratio of hyaline tail region / stylet length (eigenvalue 0.85) had the highest correlation with the first principal component. The ratio of head height/head weight (eigenvalue

0.90) and head height (eigenvalue 0.91) was best correlated with the second variable. PCA analyses showed good separation for the species of *H. avenae* and *H. filipjevi*, but revealed overlapping between the different populations of *H. avenae*.

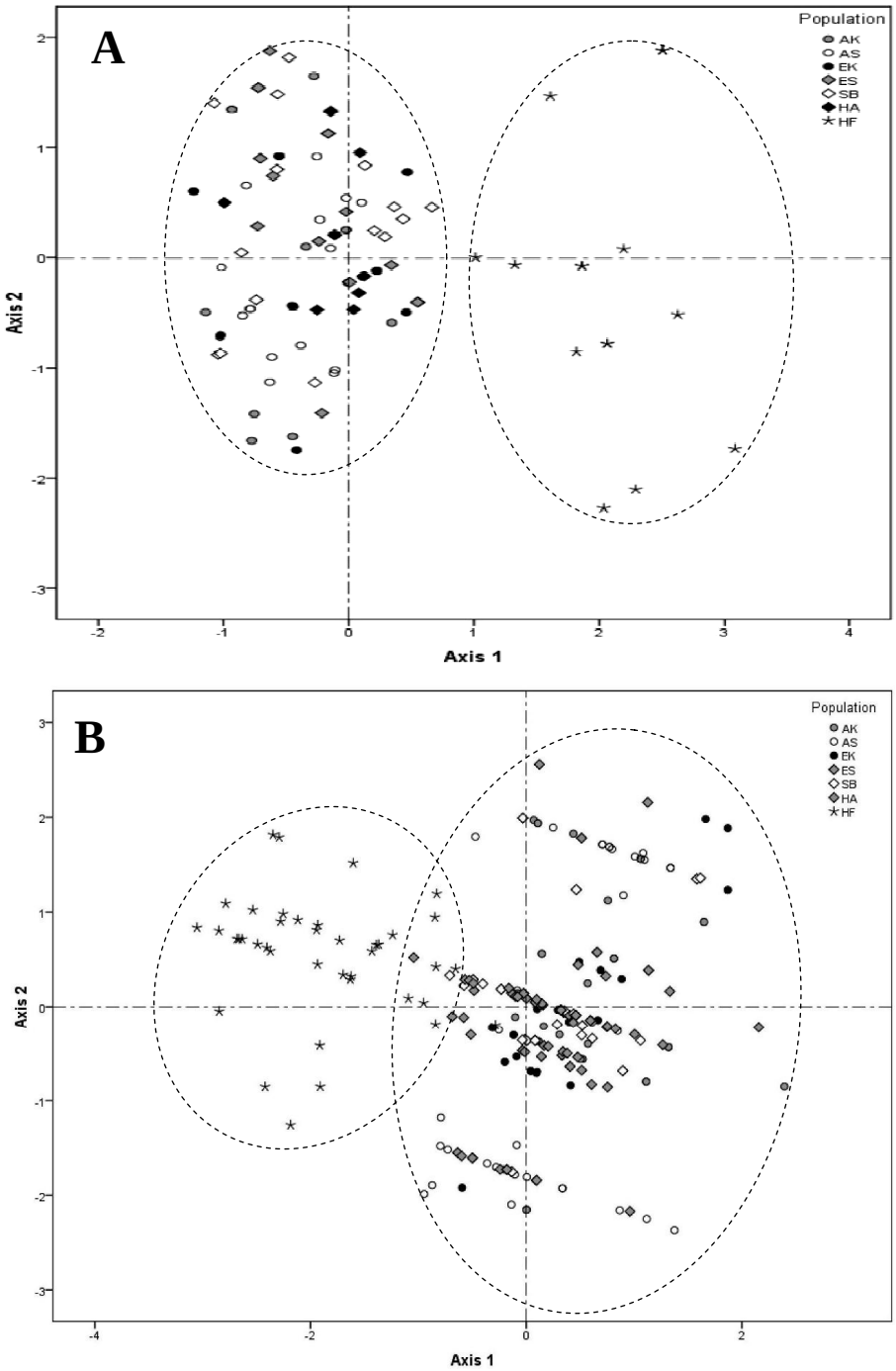

Figure 1. Two dimensional scatter plots of correlation-based PCA of morphometrical data of *Heterodera* populations **(A)** cysts vulval cone and **(B)** second stage juveniles. AK= (Abu Khalifah), AS= (Abu Suwayr), EK= (El Kasasen), ES= (El Shark), SB= (Serabeum), HA= (*H. avenae* Grafenreuth) and HF= (*H. filipjevi* Rädel).

PCR-RFLP analysis

Genomic DNA was successfully extracted from all populations and produced a single fragment of approximately 1200 bp when amplified with the primer pair 18S/26S. No PCR products were produced in the negative control without nematode DNA template. Polymorphic PCR-RFLP patterns inferred the restriction endonucleases allowed differentiation of the tested populations **(Figure 2)**. Digestion with 15 out of the 17 enzymes gave RFLPs for all studied species; *DraI and PvuII* were the only enzymes that did not restrict any of the ITS. The tested populations showed the same RFLP patterns with two restriction enzymes *Sau3AI and TruII*. Although no single enzyme could distinguish all tested species, the combination of the patterns obtained by several individual enzymes allowed differentiation of the most species and populations under study **(Table 6)**.

Twelve enzymes out of seventeen (*AluI, AvaI, Bsh1236I, HaeIII, HhaI, HinfI, Hinp1I, Hypyf3I, MspI, MvaI, RsaI* and *TaqI*) yielded RFLP profiles distinguishing *H. schachtii* (Münster) from the other populations. Four restriction enzymes (*HaeIII, HinfI, PstI* and *TaqI*) digestions allowed for the differentiation of *H. filipjevi* (Rädel) from the other populations. Digestion with these four enzymes showed unique fragments with *H. filipjevi* (Rädel) of 405.61, 858.60, 298.48 and 340.04 bp, respectively. Moreover, intraspecific polymorphism was revealed within *H. avenae* populations by *AluI and RsaI* enzymes. The restriction profiles obtained with these two enzymes clearly distinguished the German population of *H. avenae* (Grafenreuth) from the Egyptian CCN populations and from the other populations. Digestion by *AluI* and *RsaI* showed unique fragments with *H. avenae* (Grafenreuth) of 1117 bp and 1172 bp, respectively. Nevertheless, none of the tested enzymes allowed the differentiation between the surveyed Egyptian populations.

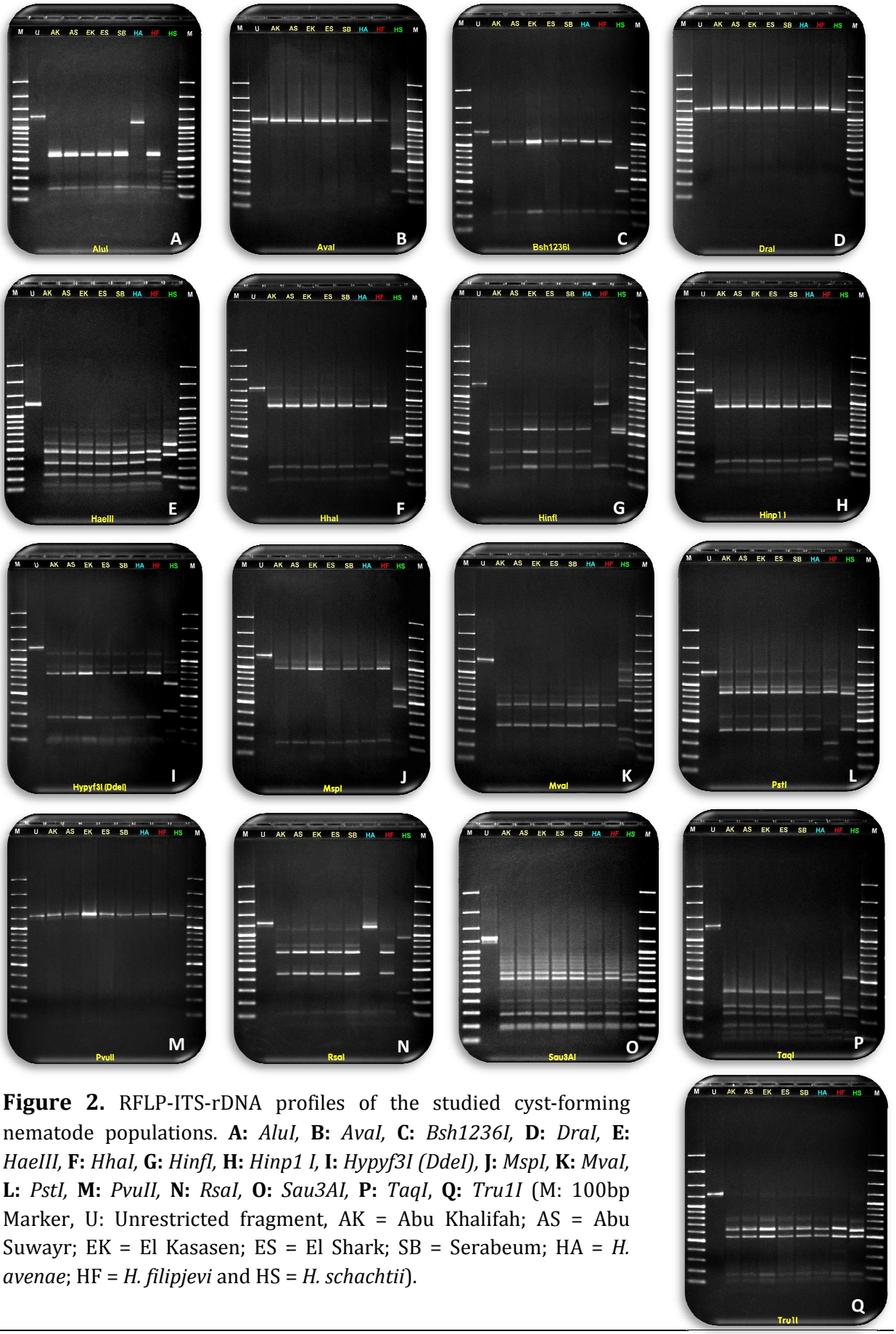

Figure 2. RFLP-ITS-rDNA profiles of the studied cyst-forming nematode populations. **A:** *AluI,* **B:** *AvaI,* **C:** *Bsh1236I,* **D:** *DraI,* **E:** *HaeIII,* **F:** *HhaI,* **G:** *HinfI,* **H:** *Hinp1 I,* **I:** *Hypyf3I (DdeI),* **J:** *MspI,* **K:** *MvaI,* **L:** *PstI,* **M:** *PvuII,* **N:** *RsaI,* **O:** *Sau3AI,* **P:** *TaqI*, **Q:** *Tru1I* (M: 100bp Marker, U: Unrestricted fragment, AK = Abu Khalifah; AS = Abu Suwayr; EK = El Kasasen; ES = El Shark; SB = Serabeum; HA = *H. avenae*; HF = *H. filipjevi* and HS = *H. schachtii*).

Table 6. Restriction fragment sizes (*bp*) of rDNA-ITS regions yielded by a single enzyme for *Heterodera* populations.

Pop.* / Enzyme	AK	AS	EK	ES	SB	HA	HF	HS
AluI	*563.27-547.83-203.64*	*565.81-547.08-206.78*	*565.76-549.64-204.53*	*565.98-550.41-206.78*	*565.93-552.58-207.49*	*1117-204.72*	*557.62-547.10-206.55*	*349.99-302.25 258.27-202.39*
AvaI	*1266*	*1263*	*1263*	*1263*	*1267*	*1264*	*1265*	*705.04-418.83-236.11*
Bsh1236I	*1051-227.60*	*1054-231.98*	*1050-230.91*	*1053-233.06*	*1053-231.75*	*1053-233.39*	*1055-232.56*	*653.76-404.40-233.39*
DraI	*1230*	*1233*	*1231*	*1229*	*1229*	*1233*	*1228*	*1228*
HaeIII	*479.17-359.77-243.77*	*480.76-360.30-246.20*	*489.56-360.06-246.84*	*481.26-360.06-246.41*	*482.65-358.16-246.33*	*489.81-370.33-247.59*	*512.28-405.61-246.89*	*637.89-470.84-197.59*
HhaI	*901-238.41-147.28*	*905-238.86-151.76*	*903-238.86-151.02*	*902-238.86-154.03*	*905-240.04-151.75*	*905-240.05-152.85*	*904-241.11-149.79*	*469.26-428.23-187.58*
HinfI	*528.62-318.93-205.39*	*526.99-320.57-205.39*	*526.55-318.88-202.79*	*527.72-320.76-205.40*	*528.17-321.32-205.39*	*528.63-320.57-206.23*	*858.60-207.10*	*522.95-487.76-207.10*
Hinp1I	*894.61-225.32-136.28*	*900-226.66-134.74*	*897.74-227.23-135.37*	*895.46-226.85-135.34*	*901-227.81-135.39*	*902-226.65-135.40*	*899.52-226.86-137.39*	*457.02-412.46-174.53*
Hypyf3I	*754.43-272.01-94.47*	*757.58-273.25-95.74*	*756.87-274.17-96.28*	*756.16-274.17-95.74*	*757.54-273.26-96.25*	*756.18-275.94-95.74*	*755.85-274.17-95.73*	*607.37-326.82-156.15*
MspI	*985-166.32*	*984-165.95*	*975-167.77*	*984-164.36*	*986-166.90*	*984-166.36*	*986-166.02*	*645.25-441.51*
MvaI	*541.48-345.83*	*544.93-347.48*	*544.21-346.57*	*543.22-345.83*	*544.93-347.50*	*544.45-347.72*	*544.45-345.81*	*1085-890.25 795.59-602.06 311.52-235.75*
PstI	*864.33-410.54*	*867.92-412.57*	*866.15-412.57*	*868.59-413.24*	*866.51-412.79*	*865.79-412.12*	*866.53-298.48-190.53*	*867.52-413.90*
PvuII	*1276*	*1276*	*1281*	*1277*	*1278*	*1277*	*1278*	*1279*
RsaI	*716.36-451.22-128.48*	*713.62-453.68-128.63*	*716.70-452.67-127.81*	*714.97-453.88-130.27*	*713.62-453.88-130.27*	*1172-131.91*	*714.27-454.10-130.17*	*955-274.55*
Sau3AI	*688.34-623.50 290.83-200.74*	*691.56-627.40 292-198.97*	*693.49-627.40 292.96-199.77*	*693.93-626.09 292.61-198.97*	*694.10-626.07 292.06-199.42*	*694.27-627.13 291.88-198.97*	*691.56-627.13 293.14-198.97*	*688.63-623.22 291.35-198.9*
TaqI	*380.49-274.69 230.75-153.15 113.55-89.44*	*382.45-273.45 229.88-152.87 112.38-89.25*	*382.28-272.28 228.66-152.46 113.88-90.13*	*380.98-272.65 228.43-153.55 118.76-90.10*	*381.90-272.94 230.46-153.29 115.42-90.11*	*382.45-276.67 228 - 153.83 116.97-90.11*	*340.04-276.82-228.11 116.54-90.12*	*520.88-266.91-224.07 115.41-88.92*
Tru1I	*623.19-534.43 162.41-101.14*	*622.52-532.07 162.39-102.91*	*622.48-532.59 160.31-102.82*	*623.55-533.13 158.53-104.41*	*623.11-533.92 162.63-106.38*	*623.35-533.90 164.94-106.31*	*622.48-530.49 162.65-107.96*	*621.75-525.78 161.50-110.58*
No. of Scored Fragments	*46*	*46*	*46*	*46*	*46*	*44*	*45*	*52*

* AK = Abu Khalifah; AS = Abu Suwayr; EK = El Kasasen; ES = El Shark; SB = Serabeum; HA = *H. avenae;* HF = *H. filipjevi* and HS = *H. schachtii.*

A total of 371 scored fragments were obtained with seventeen enzymes and used for similarity coefficients (JACCARD) analysis based on bands molecular sizes. A dendrogram **(Figure 3)** constructed from unweighted pair-group method using arithmetic averages (UPGMA) was applied to clustering the populations at different levels on a scale of similarity. Distribution of different populations mapped on the dendrogram displayed four main clusters supported by bootstrap values above 89% and relevant to each tested species and populations. The highest genetic distance (d = 0.671) and dissimilarity (s = 32.9%) was observed between the out-group *H. schachtii* and *H.avenae* group. Cluster I contained *H. schachtii* (Münster) with 100% confidence. The genetic distance and dissimilarity inside the *H.avenae* group populations decreased when the Egyptian populations and other species and populations were compared (d = 0.195 and s = 80.9% with *H. filipjevi* Rädel) (d = 0.095 and s = 90.5% with *H. avenae* Grafenreuth). Cluster II contained *H. filipjevi* (Rädel) with 98% confidence while, cluster III contained *H. avenae* (Grafenreuth) with 100% confidence. However, the lowest genetic distances (d ≤ 0.01) and highest similarity (s ≥ 98.2%) were noticed amongst the surveyed Egyptian populations. Cluster IV contained the Egyptian populations with confidence above 89%.

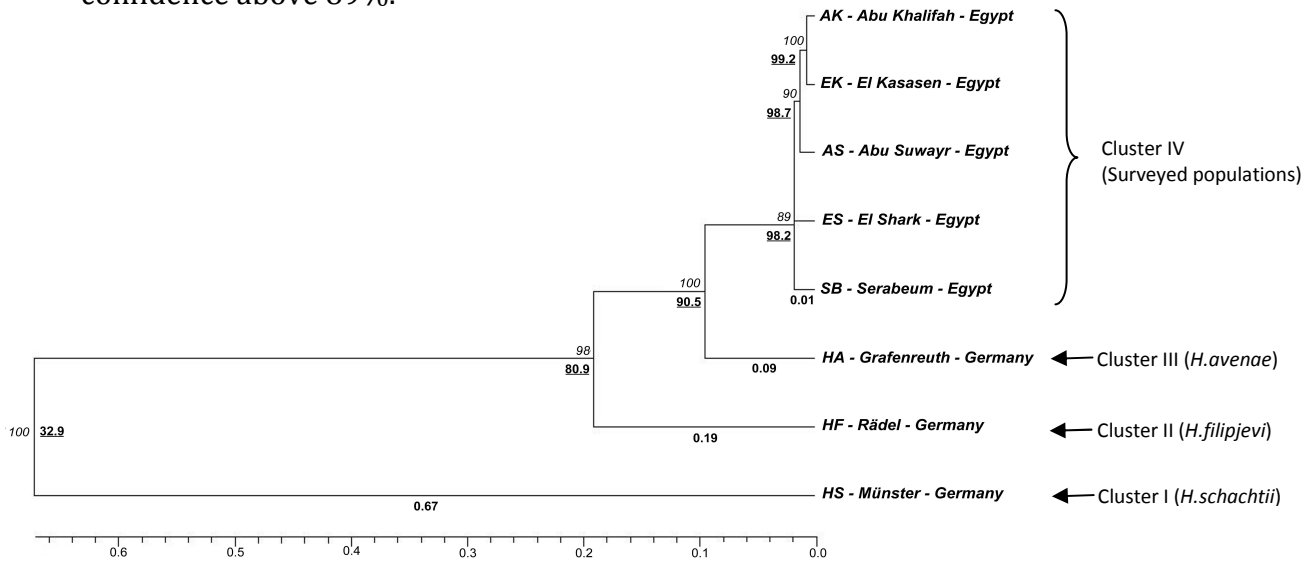

Figure 3. UPGMA dendrogram clustering the populations based on scale of similarity (underline) and Jaccard's genetic distance (**bold**) calculated from PCR-RFLP data. Bootstrap values (%) based on 1000 resampling are given on appropriate clusters (*Italic*). AK = Abu Khalifah; AS = Abu Suwayr; EK = El Kasasen; ES = El Shark; SB = Serabeum; HA = *H. avenae*; HF = *H. filipjevi* and HS = *H. schachtii*).

Sequence and Phylogenetic Analyses

The total length of the alignment including 29 sequences (8 new and 21 known ITS sequences, **Table 2**) was 1293 bp with 91.6% pairwise identity and 76.4% identical sites. The ITS region length in the outgroup taxa (*H. schachtii* group) ranged from 1002 to 1116 bp while the ITS region length in the ingroup taxa (*H.avenae* group) ranged from 964 to 1267 bp. A consensus Neighbor-Joining tree clustering the species and the populations at different levels based on genetic distance was constructed from the ITS sequence alignment (**Figure 4**). The tested populations were distributed in the tree within four clusters in two main groups supported by bootstrap values above 80%. The first main group was *H.avenae* group which considered as ingroup taxa and included three clusters. Cluster I contained the European populations of *H. avenae* with 82.2% bootstrap value, while cluster II contained the African and the Asian populations of *H. avenae* with 97.9% bootstrap value. Cluster III contained *H. filipjevi* populations with 93% bootstrap value. On the other hand, the second main group was *H. schachtii* group which contained several populations of *H. schachtii* with 100% bootstrap value and considered as a outgroup taxa.

The sequences divergence between outgroup and ingroup taxa ranged from 16 to 18% while sequences divergence inside the outgroup taxa was ranged from 0.5 to 1.8%. On the other hand, sequences divergence inside the ingroup taxa was ranged from 0 to 2.5%. The highest sequences divergence inside the ingroup taxa was observed between cluster I (European populations of *H. avenae*) and III (*H. filipjevi* populations) with sequences divergence ranged from 2 to 2.5% while the lowest sequences divergence was detected in a range from 0.2 to 0.9% between cluster I (European populations of *H. avenae*) and II (African and the Asian populations of *H. avenae*). In addition to, sequences divergence from 1.9 to 2.3% was found between cluster II and III.

Cluster I in the ingroup taxa clustered the new sequence of *H. avenae* (Grafenreuth pop.) with the other European sequences of *H. avenae* from the GenBank with sequences divergence ranged from 0 to 0.4%. *H. avenae* population from Spain showed the highest sequences divergence from Grafenreuth population while the German and

the English populations of *H. avenae* showed high similarities to the new sequence of Grafenreuth population. The new sequences of the Egyptian populations of *H. avenae* showed high similarities to each others with sequences divergence ranged from 0 to 0.2% and these sequences were clustered in the ingroup taxa (cluster II) with the Asian sequences of *H. avenae* from the GenBank with sequences divergence ranged from 0 to 0.5%. *H. avenae* populations from India and Saudi Arabia showed the highest sequences divergence from the Egyptian populations while populations from Iran, Israel and China showed the lowest sequences divergence to the new Egyptian sequences.

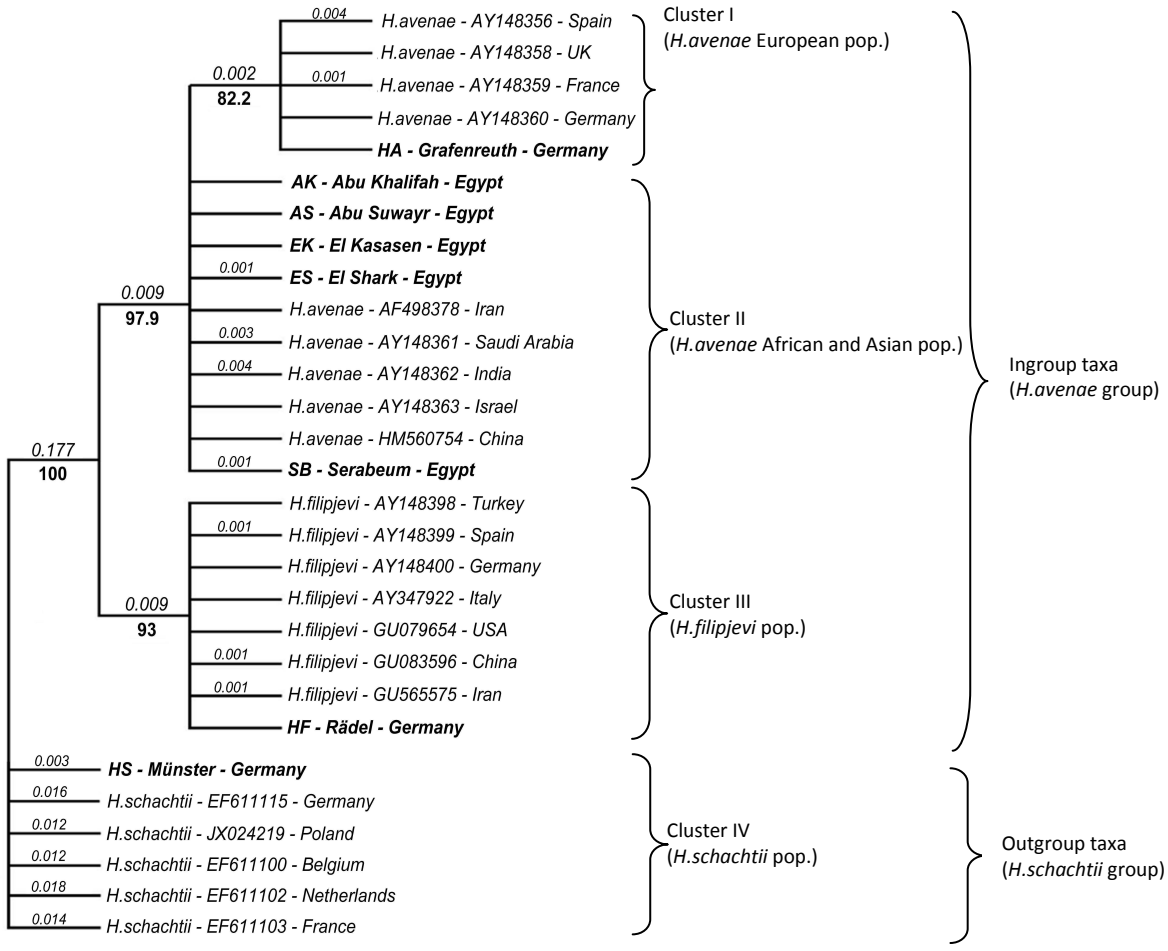

Figure 4. Neighbor-joining consensus tree constructed from the ITS sequence alignment for 29 species of cyst-forming nematodes. Bootstrap values (%) based on 1000 resampling (**bold**) and genetic distances (*italic*) are given in the appropriate clusters. New sequences are indicated by bold. See **Table 2.** for origin of nematode sequences.

DISCUSSION

This study provides new information on the occurrence and distribution of *H. avenae* in major wheat growing regions in Northern Egypt. This is the first report detecting *H. avenae* infecting wheat in Ismailia province and Sinai. The highest incidence of *H. avenae* was recorded in the area with the most intensive cultivation of wheat, El Shark location (West Sinai) **(Table 3)** where wheat had been cultivated continuously. Short cereal rotations in the temperate semiarid regions favor nematode population development and associated yield loss **(Sikora 1987)**. *H. avenae* was found infecting wheat fields in Nile Delta, Alexandria and El-Behera Governorates in Northern Egypt at an incidence of 38% **(Ibrahim and Handoo, 2007)**. Higher prevalence of *H. avenae* was recorded in our study which indicates that *H. avenae* populations may increase more in light well-draining soils of Ismailia and Sinai than the heavy soil of Nile valley area. In **1984, Brown** reported that *H. avenae* was detected in sandy soil in Victoria and South Australia, but not in heavy soils in the same area which may support our observation.

Morphologically, two well differentiated groups were classified **(Figure 2)**. The first group is represented by *H. avenae* and the second is represented by *H. filipjevi*. The morphological characters for distinguishing *H. avenae* from *H. filipjevi* were the presence of distinct underbridge in the vulval cone and small bullae situated below the fenestrae of cysts of *H. filipjevi*, as opposed to the absence of an underbridge and the presence of well-developed bullae surrounding the vulval cone of the cysts of *H. avenae* as described by **Rivoal *et al.,* (2003)** and **Subbotin *et al.,* (2003)**. The morphometric characters, such as hyaline part of the tail of the second stage juveniles and the fenestra length of cysts, are also separating these species **(Madzhidov, 1981; Valdeolivas and Romero, 1990)**.

The rDNA intergenic spacer region has been useful for diagnostics and phylogenetic relationships between isolates and taxa of plant parasitic nematodes **(Ferris *et al.,* 1993 and 1994)**. A comparative analysis of several populations of different species of the *H. avenae* group was done by **Subbotin *et al.,* (1999)** and

showed that rDNA-RFLPs can distinctly separate species and populations within the *H. avenae* group. These methods have likewise been adopted in subsequent more geographically-focused studies **(Maafi *et al.,* 2003; Madani *et al.,* 2004; Yan and Smiley 2010)**, and further investigation of CCN in Egypt using such approaches would build on these data.

Three divergent clusters in the complex of *H. avenae* group were readily observed from our PCR-RFLP analysis. The first cluster was composed of the Egyptian populations of *H. avenae*; the second includes the German population of *H. avenae* and the third cluster represented by *H. filipjevi*. Four restriction enzymes (*HaeIII, HinfI, PstI* and *TaqI*) digestions allowed for the differentiation of *H. filipjevi* from different types of *H. avenae* populations. This result was in harmony with the finding of **Yan and Smiley (2010)** that two enzymes (*TaqI and HaeIII*) separated *H. filipjevi* and *H. avenae* from *H. schachtii* and six restriction enzymes (*TaqI, HinfI, PstI, HaeIII, RsaI, and AluI*) allowed for distinguishing between *H. filipjevi* and *H. avenae*. **Bekal *et al.,* (1997)** found that digestion with *PstI* clearly separated *H. filipjevi* from *H. avenae*.

Molecular polymorphism has frequently been observed between geographically isolated populations of *H. avenae*. **Subbotin *et al.,* (2003)** reported that restriction by *AluI* and *RsaI* allowed distinction of the following *H. avenae* populations: i) European populations (Type A) – no restriction by *AluI* and a fragment more than 1000 bp long after restriction by *RsaI*, ii) Asian populations (Type B) – PCR product restricted by *AluI* and *RsaI*, iii) French populations (Type C), iv) Moroccan population (Type D). This finding is partially in agreement with our results which showed that the restriction profiles obtained with *AluI* and *RsaI* enzymes distinguished the German population (Grafenreuth) from the Egyptian populations of *H. avenae*. However, the Grafenreuth population in our results showed a different RFLP profile (two fragments after restriction by *AluI* and *RsaI*) than the European populations (Type A) in the reports of **Subbotin *et al.,* (1999 and 2003)**. This variation may arise from the amplification of a longer part of the ribosomal DNA, 1200 bp in our study instead of 1060 bp, suggesting that the targeted restriction site was located in the supplement amplicon. *AluI* enzyme

reveals heterogeneity of the ITS region among several populations of *H. avenae* **(Maafi *et al.,* 2003)**.

The Egyptian populations of *H. avenae* were restricted by *AluI* and *RsaI* enzymes and were related to *H. avenae* populations belonging to Type B. Similar results were recorded in nearby countries; **Zheng *et al.,* (2000)** mentioned that Bet-Dagan populations of *H. avenae* from Israel were belonging to ITS type B with the Asian populations of *H. avenae*. Additionally, *H. avenae* type B is widely spread in wheat growing areas in Southwest of Iran **(Ahmadi and Tanha Maafi, 2009)**. The Chinese CCN populations were classified by **Peng *et al.,* (2009)** as type B. The Indian population of *H. avenae* (Hav8) was identified as a type B **(Subbotin *et al.,* 1999)**. **Imren *et al.,* (2012)** also identified some populations from Mardin in Southeast Anatolia of Turkey as *H. avenae* type B.

A consensus Neighbor-Joining tree constructed from the ITS sequence alignment, clustered the *H. avenae* group species and the populations at different levels based on the genetic distance **(Figure 3)**. These results are in agreement with **Subbotin *et al.,* (2003)** who found that the ITS sequences of the *H. avenae* group were distributed in the same way like our results, within four clades in two main groups. The first main group considered as *H. avenae* group included three clades: i) populations from Africa and Asia, ii) several populations from France, iii) other populations from Europe. **Bekal *et al.,* (1997)** hypothesized that the most likely site of origin of *H. avenae* is within the cereal areas in the Middle East and that this nematode could have been introduced to West Asia and Australia. **Hesar *et al.,* (2012)** mentioned that the phylogenetic relationships within the '*H. avenae* group' inferred from analyses of the full ITS data set clustered the Iranian population of *H. avenae* with the populations from India and Israel while the European populations of *H. avenae* from Germany, France and Spain were clustered together.

This is the first report of *H. avenae* populations on wheat in Ismailia province and West Sinai of Egypt. The concurrence between our morphometric and molecular data is supported by previous reports that revealed the strength of the relative relationship

between morphometric and molecular traits **(Reed and Frankham, 2001; Rivoal *et al.,* 2003; Subbotin *et al.,* 2003)**. This study showed the presence of *H. avenae* type B in Northern Egypt, highlighting its morphological and molecular characteristics. This report illustrated that even *H. avenae* has not been recognized as a quarantine organism, monitoring its occurrence and spread at the domestic and international levels appears to be an urgent need since it is in an active state of evolution and in the process of speciation which is exhibited in the form of large intraspecific variability. The genetic variation presented in this study between the Egyptian and the German populations of *H. avenae* may improve detection and identification among different geographical populations of cereal cyst nematode.

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CHAPTER 3

Influence of temperature and storage conditions on the hatching behavior of cereal cyst nematode (*Heterodera avenae* Wollenweber) from Egypt

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ABSTRACT

The cereal cyst nematode *Heterodera avenae* Wollenweber, has been reported in wheat fields in Egypt; however no information is available on the hatch of these populations of *H. avenae*. The aim of this study was to subject five Egyptian populations from different regions in Ismailia province to different temperatures and storage durations to determine their effect on the juvenile emergence. The Egyptian populations were compared to one population originating from a different climatic zone (Grafenreuth, Bavaria, Germany). The results showed that the hatching pattern of *H. avenae* populations from different areas of Ismailia, Egypt did not differ from each other. Although the Egyptian and the German populations of *H. avenae* hatched primarily between 5 and 20°C, significant differences in the hatching have been observed. Highest emergence of juveniles of the Egyptian populations was observed between 10°C and 15°C, whereas the highest level of juvenile's emergence of the German population was between 5° and 10°C. Subjection of cysts to different storage temperatures to simulate seasonal variations was used to further investigate the hatching behavior of the studied populations. Exposing cysts to 5°C before incubation at 10°C stimulated the hatch of the German population of *H. avenae* significantly compared to the control. Whereas, exposing cysts to 30°C inhibited and delayed the hatch of the Egyptian populations of *H. avenae* after incubating the cysts at 10°C. Moreover, exposing cysts to 20°C stimulated the hatch of the Egyptian populations of *H. avenae* significantly compared to the control after incubating the cysts at 10°C. The hatching behavior of the Egyptian populations of *H. avenae* was in parallel to the Mediterranean ecotypes which have winter activity, while the hatching pattern of the German population was of the Northern ecotypes with spring activity. Control strategies such as early planting, minimum tillage and rotation that are effective against the Mediterranean ecotype of *H. avenae* in southern Australia, France and Spain could be applied or developed for the Egyptian populations of *H. avenae*.

Keywords - *Heterodera avenae*, Hatching, Temperature, Storage period, Mediterranean ecotype.

INTRODUCTION

The cereal cyst nematode (CCN) *Heterodera avenae* Wollenweber, has a global distribution and has been found in most of the countries where cereals are cultivated. It is one of the most economically important plant parasitic nematode attacking temperate cereals, including wheat and barley **(Sikora 1987)**.

The success of this nematode is due, in part, to the synchronization between nematode and host life cycle. The hatching of second stage juveniles is an important part of this synchronization. The influence of temperature on the hatching of eggs and the emergence of second stage juveniles of *H. avenae* has been reviewed by many workers under laboratory and field conditions. Based on the geographic origin of the populations, two ecotypes of *H. avenae* can be differentiated. These ecotypes differ in their hatching periodicity **(Evans and Perry, 1976; Rivoal, 1982)**. One ecotype (Northern ecotype) from the temperate oceanic climatic zone in North-Western Europe, Great Britain **(Kerry and Jenkinson, 1976)** and Northern France **(Rivoal, 1978)** mainly hatches in spring. The other ecotype (Mediterranean ecotype) from the Mediterranean climate, such as Italy **(Greco, 1981)**, the south of France **(Rivoal, 1978)** and Israel **(Mor et al., 1992)** mainly hatches in winter. The latter ecotype has also been reported from Australia **(Meagher, 1970)**.

Although, numerous experiments have been made to figure out the temperature requirements for hatching of *H. avenae* in several geographic areas, there is no information on the influence of temperature on the hatching and emergence of juveniles of Egyptian populations of *H. avenae*. In this study, five Egyptian populations were subjected to different temperature and storage periods to determine their ecotype and temperature requirements. The Egyptian populations were compared to one population originating from a different climatic area (Grafenreuth, Bavaria, Germany). Hatching was studied at different temperatures simulating seasonal variations in the two different climatic areas.

This study is valuable for the production and availability of juveniles, which is a requirement for the investigations on *H. avenae* as work is often restricted by lack of juveniles. Furthermore, the results from this study may be useful for the development of control strategies to *H. avenae.* Early planting, minimum tillage and rotation that are effective against the Mediterranean ecotype of *H. avenae* in southern Australia, France and Spain could be applied or developed for the Egyptian populations of *H. avenae*.

MATERIALS AND METHODS

Nematode populations

Cysts of five Egyptian populations were compared to one population from Germany **(Table 1)**. These populations were identified as *H. avenae* based on morphology, PCR-RFLP and rDNA-ITS sequence analyses (Chapter 2). Cysts of all populations were dried at room temperature (20±2°C) and kept at 7°C and subsequently reared on the susceptible Egyptian wheat cultivar (*Triticum aestivum*) cultivar 'Sakha 93' for further use in experiments. Cysts were extracted from the soil using the floatation technique **(Shepherd, 1986)**. Counting and separation of cysts from soil debris and other organic materials retained on the filter paper were carried out at 25x magnification under a stereoscopic binocular (Leica MZ8). Newly-formed cysts were used in the experiments.

Table 1. Origin and cyst content of *Heterodera avenae* populations used in this study.

Code	Location	Cyst contents	Country
AK	Abu Khalifah region, Ismailia	188 ± 15.2	Egypt
AS	Abu Suwayr region, Ismailia	190 ± 12.7	Egypt
EK	El Kasasen region, Ismailia	199 ± 11.2	Egypt
ES	El Shark region (West Sinai), Ismailia	198 ± 12.0	Egypt
SB	Serabeum region, Ismailia	195 ± 13.9	Egypt
GR	Grafenreuth, Bavaria	193 ± 14.2	Germany

Hatching of *H. avenae* at different temperatures

The effect of different constant temperatures on the emergence of second stage juveniles of six *H. avenae* populations **(Table 1)** was investigated using six incubators (Heraeus BK 5060 EL) set at 30, 25, 20, 15, 10 and 5°C, respectively. Five replicates per treatment were used; each replicate consisted of five newly-formed cysts placed in 1.0 ml tap water in a 2.0 ml Eppendorf tube. The tubes were arranged in Styrofoam boxes and placed at the respective temperatures.

Hatching of *H. avenae* following storage at different temperatures

The influence of different storage temperatures applied for different periods of time on subsequent hatch of six *H. avenae* populations **(Table 1)** was studied. Cysts of *H. avenae* populations were stored for three different periods of 4, 8 or 12 weeks at temperatures of 30, 20 or 5°C. There were five replicates per treatment; each replicate consisted of five newly-formed cysts in a 2.0 ml Eppendorf tube. The tubes were arranged in boxes and placed in the respective incubators. At the end of the storage period, one ml of tap water was added to each Eppendorf tube and the tubes were placed at constant temperature of 10°C for 12 weeks. These treatments were compared with a control treatment incubated directly at 10°C without previous storage period.

Data collection and analysis

During the incubation period in both experiments, hatched second stage juveniles from each tube were removed and counted weekly under a compound microscope (Axiovert 25) at 40 x magnifications. The tubes were immediately replenished with 1.0 ml tap water per tube and returned to the incubator. This procedure was repeated at weekly intervals for 12 weeks. At the end of the experiment, the numbers of unhatched eggs were counted after squashing the cysts according to **Seinhorst and Den Ouden (1966)** and the total number of eggs (hatched plus unhatched eggs) per replicate was determined. Levene's test was used to test the homogeneity of variance. Percentages of cumulative hatched juveniles at each sampling date in both experiments were analyzed using ANOVA (SPSS version 19.0, IBM Corporation, New Orchard Road Armonk, New York, United States). Means were separated using Tukey HSD test at $P \leq 0.05$.

RESULTS

Hatching of *H. avenae* at different temperatures

Percentages of cumulative hatched juveniles of six *H. avenae* populations were recorded over 12 weeks at temperatures of 30, 25, 20, 15, 10 and 5°C **(Table 2)**. No second stage juveniles emerged from the incubated cysts of all tested populations at temperatures of 30 and 25°C. No significant differences in the hatching pattern were observed among the Egyptian populations at different temperature treatments, while the German population showed a hatching pattern different from the Egyptian populations.

At 20°C, the Egyptian and the German populations started to hatch at different times. All Egyptian populations hatched from the first week after the start of the test while the German population only began to hatch after six weeks **(Table 2)**. In despite of the early beginning of hatch in the Egyptian populations, the rate of hatch at 20°C was the lowest for all populations compared to the other temperature treatments **(Figure 1)**. The percentages of final cumulative hatch of the Egyptian populations were comparable and ranged between 26.9 – 32.3%. After 12 weeks, the cumulative hatch of juveniles of the German population was significantly lower (13.9%) than that of the Egyptian populations.

At 15°C, second stage juveniles of all tested populations emerged from cysts in the second week and their percentages of final cumulative hatch were higher than those observed at 20°C **(Figure 1)**. The percentages of final cumulative hatch of the Egyptian populations were comparable and ranged between 45.1 – 54.9%, while the German population showed the lowest percentage of hatching of 37.9% after 12 weeks.

Table 2. Percentage of cumulative hatched juveniles (± standard deviation) of *H. avenae* populations over 12 weeks at different temperatures.

Temp.	Pop.*	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week12
20°C	AK	0.5 ± 0.5 **a**	2.0 ± 0.8 **ab**	4.0 ± 0.9 **a**	6.5 ± 1.1 **a**	9.7 ± 1.4 **a**	15.8 ± 1.3 **ab**	20.1 ± 2.0 **a**	22.9 ± 2.2 **a**	25.2 ± 2.4 **a**	26.9 ± 2.6 **a**	28.3 ± 2.9 **a**	29.2 ± 3.2 **a**
	AS	0.2 ± 0.3 **a**	2.0 ± 0.8 **ab**	4.0 ± 1.1 **a**	6.2 ± 1.4 **a**	9.5 ± 0.8 **a**	14.6 ± 1.2 **ab**	20.4 ± 2.1 **a**	22.7 ± 2.2 **a**	24.7 ± 2.5 **a**	26.6 ± 2.8 **a**	28.4 ± 3.1 **a**	29.8 ± 3.5 **a**
	EK	0.3 ± 0.2 **a**	1.9 ± 1.1 **ab**	4.1 ± 1.7 **a**	7.1 ± 2.0 **a**	10.8 ± 2.4 **a**	15.9 ± 3.1 **ab**	21.4 ± 3.8 **a**	24.9 ± 4.1 **a**	27.0 ± 4.2 **a**	28.8 ± 4.4 **a**	30.1 ± 4.2 **a**	30.7 ± 4.1 **a**
	ES	0.7 ± 0.2 **a**	2.1 ± 0.4 **a**	4.3 ± 1.0 **a**	7.0 ± 1.8 **a**	10.2 ± 2.7 **a**	17.7 ± 2.5 **a**	23.0 ± 3.0 **a**	26.4 ± 3.4 **a**	29.1 ± 3.9 **a**	31.0 ± 4.1 **a**	32.0 ± 4.2 **a**	32.3 ± 4.3 **a**
	SB	0.2 ± 0.3 **a**	0.7 ± 0.5 **bc**	2.6 ± 0.8 **ab**	5.2 ± 1.1 **a**	8.2 ± 1.8 **a**	13.6 ± 2.7 **b**	17.8 ± 3.5 **a**	20.9 ± 4.1 **a**	23.2 ± 4.5 **a**	24.9 ± 4.9 **a**	26.1 ± 5.0 **a**	26.9 ± 5.2 **a**
	GR	0 ± 0 **a**	0 ± 0 **c**	0 ± 0 **b**	0 ± 0 **b**	0 ± 0 **b**	0.1 ± 0.1 **c**	1.2 ± 0.2 **b**	2.9 ± 0.6 **b**	4.7 ± 1.0 **b**	7.3 ± 0.9 **b**	10.4 ± 0.7 **b**	13.9 ± 0.9 **b**
15°C	AK	0 ± 0 **a**	2.7 ± 1.5 **a**	10.8 ± 2.7 **a**	19.4 ± 4.0 **a**	23.7 ± 4.9 **a**	27.3 ± 5.6 **a**	30.1 ± 5.3 **a**	34.0 ± 5.3 **a**	39.0 ± 6.5 **a**	42.2 ± 6.6 **a**	45.1 ± 6.6 **a**	47.4 ± 6.4 **ab**
	AS	0 ± 0 **a**	0.5 ± 0.6 **b**	3.9 ±2.0 **b**	13.3 ± 2.1 **b**	19.1 ± 1.9 **ab**	23.9 ± 1.4 **ab**	27.7 ± 0.8 **a**	32.8 ± 1.5 **a**	39.9 ± 1.8 **a**	45.7 ± 1.0 **a**	50.6 ± 1.3 **a**	54.9 ± 2.5 **a**
	EK	0 ± 0 **a**	1.4 ± 0.5 **ab**	5.5 ± 1.5 **b**	12.0 ± 1.4 **b**	17.3 ± 1.6 **bc**	20.4 ± 2.0 **abc**	25.6 ± 1.8 **a**	31.6 ± 2.0 **a**	38.9 ± 2.3 **a**	45.5 ± 2.5 **a**	51.2 ± 2.1 **a**	54.7 ± 2.6 **a**
	ES	0 ± 0 **a**	1.4 ± 0.5 **ab**	3.6 ± 1.1 **b**	9.8 ± 2.1 **b**	15.4 ± 3.4 **bc**	19.2 ± 3.7 **bc**	22.6 ± 4.3 **ab**	26.8 ± 5.2 **ab**	32.3 ± 5.4 **ab**	37.2 ± 5.2 **ab**	41.5 ± 5.1 **ab**	45.1 ± 5.3 **ab**
	SB	0 ± 0 **a**	0.9 ± 0.6 **b**	4.9 ± 1.6 **b**	10.6 ± 2.6 **b**	14.9 ± 3.5 **bc**	18.9 ± 4.8 **bc**	23.2 ± 5.2 **ab**	28.0 ± 5.7 **ab**	34.2 ± 5.9 **ab**	39.9 ± 6.4 **a**	45.3 ± 7.4 **a**	50.6 ± 8.7 **a**
	GR	0 ± 0 **a**	0.9 ± 0.4 **ab**	4.1 ± 0.6 **b**	9.5 ± 1.9 **b**	13.0 ± 2.3 **c**	15.3 ± 3.1 **c**	17.7 ± 3.3 **b**	20.5 ± 3.7 **b**	26.1 ± 4.3 **b**	30.6 ± 5.0 **b**	34.6 ± 5.4 **b**	37.9 ± 5.8 **b**
10°C	AK	0 ± 0 **a**	1.6 ± 1.1 **a**	6.5 ± 1.8 **a**	13.2 ± 2.4 **a**	21.0 ± 3.2 **ab**	30.5 ± 4.3 **ab**	41.3 ± 4.0 **ab**	50.2 ± 4.3 **a**	58.2 ± 4.0 **a**	64.6 ± 3.5 **a**	69.2 ± 3.5 **a**	72.8 ± 3.4 **a**
	AS	0 ± 0 **a**	1.0 ± 1.1 **a**	5.9 ± 1.6 **a**	12.9 ± 2.4 **a**	20.6 ± 3.3 **ab**	32.1 ± 3.2 **ab**	42.8 ± 2.8 **a**	50.4 ± 3.2 **a**	57.3 ± 3.8 **a**	63.2 ± 3.6 **a**	67.0 ± 4.0 **ab**	69.8 ± 4.3 **a**
	EK	0 ± 0 **a**	1.1 ± 1.0 **a**	5.9 ± 1.9 **a**	12.6 ± 2.0 **a**	20.2 ± 2.7 **ab**	30.2 ± 2.9 **ab**	40.1 ± 2.9 **ab**	47.5 ± 3.6 **ab**	54.6 ± 3.6 **ab**	60.7 ± 3.9 **ab**	66.2 ± 5.1 **ab**	69.1 ± 4.6 **ab**
	ES	0 ± 0 **a**	1.0 ± 1.2 **a**	5.6 ± 1.7 **a**	13.0 ± 1.2 **a**	22.2 ± 1.8 **a**	32.4 ± 2.9 **ab**	42.3 ± 3.3 **a**	51.0 ± 4.1 **a**	58.1 ± 4.6 **a**	63.7 ± 4.1 **a**	69.0 ± 4.5 **a**	72.3 ± 4.0 **a**
	SB	0 ± 0 **a**	1.3 ± 1.1 **a**	5.8 ±1.7 **a**	13.2 ± 0.9 **a**	22.5 ± 1.4 **a**	32.7 ± 2.3 **a**	42.7 ± 2.7 **a**	51.4 ± 3.2 **a**	58.2 ± 4.0 **a**	63.6 ± 3.4 **a**	68.7 ± 3.6 **a**	72.1 ± 4.5 **a**
	GR	0 ± 0 **a**	1.1 ± 1.1 **a**	4.6 ± 2.0 **a**	10.2 ± 2.8 **a**	16.5 ± 3.3 **b**	25.5 ± 4.3 **b**	35.1 ± 3.2 **b**	42.7 ± 3.0 **b**	49.4 ± 2.6 **b**	55.2 ± 2.8 **b**	59.5 ± 3.4 **b**	62.0 ± 3.3 **b**
5°C	AK	0 ± 0 **a**	0 ± 0 **a**	0 ± 0 **a**	0 ± 0 **a**	0.7 ± 0.7 **a**	2.9 ± 1.5 **a**	6.4 ± 1.9 **a**	10.5 ± 2.2 **a**	14.8 ± 2.9 **a**	20.3 ± 3.5 **a**	25.9 ± 4.2 **a**	31.6 ± 5.1 **a**
	AS	0 ± 0 **a**	0 ± 0 **a**	0 ± 0 **a**	0 ± 0 **a**	0.9 ± 0.9 **a**	3.3 ± 1.5 **a**	6.5 ± 2.0 **a**	10.5 ± 2.6 **a**	14.8 ± 3.5 **a**	20.1 ± 4.4 **a**	27.2 ± 4.3 **a**	34.3 ± 4.6 **a**
	EK	0 ± 0 **a**	0 ± 0 **a**	0 ± 0 **a**	0 ± 0 **a**	1.2 ± 1.1 **a**	4.4 ± 2.3 **a**	8.3 ± 3.4 **a**	13.2 ± 4.0 **a**	18.3 ± 4.3 **a**	24.0 ± 4.9 **a**	30.8 ± 4.6 **a**	37.8 ± 4.5 **a**
	ES	0 ± 0 **a**	0 ± 0 **a**	0 ± 0 **a**	0 ± 0 **a**	0 ± 0 **a**	1.2 ± 0.9 **a**	4.6 ± 0.9 **a**	9.0 ± 0.9 **a**	13.6 ± 1.7 **a**	19.7 ± 3.1 **a**	27.4 ± 4.0 **a**	35.2 ± 5.0 **a**
	SB	0 ± 0 **a**	0 ± 0 **a**	0 ± 0 **a**	0 ± 0 **a**	0 ± 0 **a**	2.0 ± 1.2 **a**	5.9 ± 2.2 **a**	10.8 ± 2.9 **a**	16.0 ± 3.5 **a**	22.3 ± 3.8 **a**	29.5 ± 3.7 **a**	36.9 ± 3.9 **a**
	GR	0.2 ± 0.2 **a**	1.8 ± 0.8 **b**	5.9 ± 1.1 **b**	11.1 ± 1.3 **b**	17.0 ± 1.2 **b**	24.2 ± 1.5 **b**	31.0 ± 1.8 **b**	36.8 ± 2.1 **b**	42.0 ± 2.2 **b**	46.1 ± 2.3 **b**	49.0 ± 2.2 **b**	50.9 ± 2.2 **b**

*AK= Abu Khalifah, AS= Abu Suwayr, EK= El Kasasen, ES= El Shark, SB= Serabeum, GR= Grafenreuth.

Cumulative hatched juveniles were expressed as a percentage of total number of juveniles.

Cumulative hatched juveniles in a column at each temperature followed by the same letter are not significantly different according to Tukey HSD test at $P \leq 0.05$.

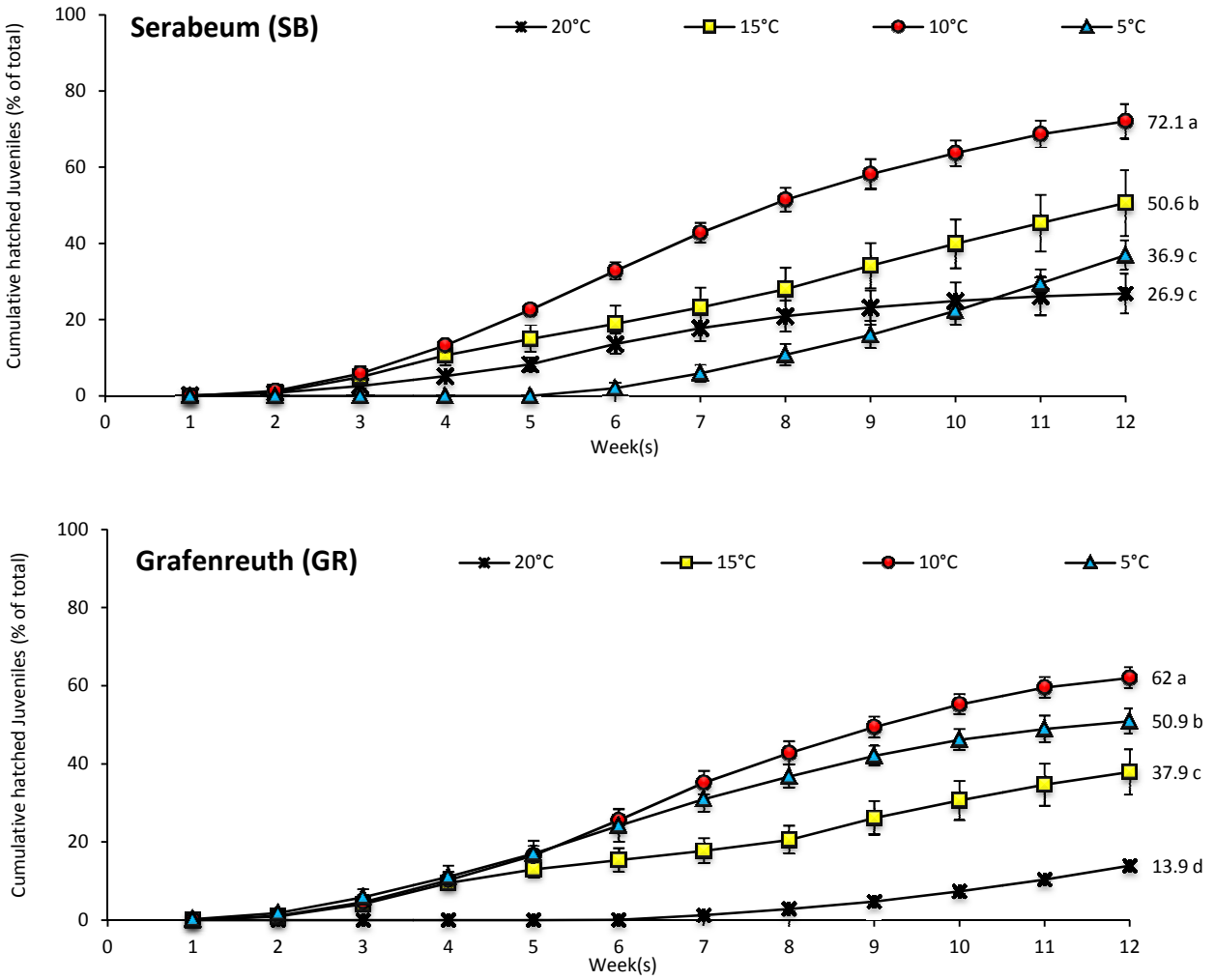

Figure 1. Effect of different temperatures on the hatch of one Egyptian population **(SB)** and one German population **(GR)** of *H. avenae*. Cumulative percentages of hatched juveniles followed by the same letter are not significantly different according to Tukey HSD test at $P \leq 0.05$. Vertical bars indicate standard deviation of the means.

At 10°C, all populations showed final cumulative hatch higher (>60%) than at all other temperature treatments after 12 weeks **(Table 2)**. Hatching of juveniles from the incubated cysts started in week two. Cumulative hatch higher than 70% was detected for three Egyptian populations AK, ES and SB and ranged between 72.1 - 72.8%. The German population had a lower cumulative hatch (62%) than the other populations. The cumulative hatch of populations AS and EK were 69.8 and 69.1%, respectively.

At 5°C, the German population showed significantly higher cumulative hatch than the Egyptian populations **(Table 2)**. Hatching of the German population took place from the first week after the start of the test while beginning of hatch of the Egyptian populations started only six weeks after the beginning of the experiment. The percentages of final cumulative hatch of the Egyptian populations ranged between 31.6 – 37.8% while the emergence of juveniles of the German population was 50.9% after 12 weeks. In general, hatching at 5°C was higher than at 20°C for all tested populations but was lower than hatching at 10°C **(Figure 1)**.

Hatching of *H. avenae* after storage at different temperatures

Percentages of cumulative hatched juveniles of six *H. avenae* populations **(Table 1)** were recorded at 10°C over 12 weeks following prior exposure to different storage temperatures of 30, 20 or 5°C. At each storage temperature the cysts were stored for 0, 4, 8 or 12 weeks. Incubation at 10°C following prior exposure to different storage periods was chosen because at this temperature all populations showed higher hatch than at all other constant temperature treatments after 12 weeks in the previous hatching experiment. Different pre-treatments at 30, 20 and 5°C which were aimed to stimulate temperature conditions of summer, spring (or autumn) and winter, respectively. No significant differences in the hatching pattern were observed amongst the Egyptian populations at different temperature treatments.

Storage of cysts of *H. avenae* populations at 30°C for 4, 8 or 12 weeks before incubation at 10°C for 12 weeks, had similar effects on the hatching patterns of all tested populations **(Table 3, Figure 2)**. Storage at 30°C resulted in a significant decrease and delay in the hatching of all populations, compared to the hatch from cysts that had not been exposed to this temperature. Delayed and reduced hatch was positively correlated with increasing the storage periods at 30°C.

Table 3. Influence of storage temperature at 30°C for different periods (0, 4, 8 and 12 weeks) prior incubation at 10°C over 12 weeks, on the cumulative hatch of *H. avenae* populations.

Pop.*	Storage period	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week12
AK	0	0 ± 0 **a**	2 ± 1.1 **a**	7.1 ± 1.6 **a**	14.4 ± 2.3 **a**	22.7 ± 3 **a**	31.5 ± 4 **a**	42.1 ± 3.7 **a**	50 ± 4 **a**	57.6 ± 3.7 **a**	64.2 ± 3.2 **a**	69.5 ± 3.2 **a**	74.2 ± 3.2 **a**
	4	0 ± 0 **a**	0 ± 0 **b**	2.8 ± 0.9 **b**	6.5 ± 1.1 **b**	11.3 ± 1.6 **b**	17 ± 1.8 **b**	25.2 ± 1.4 **b**	31.8 ± 1.4 **b**	38 ± 1.9 **b**	43.9 ± 2.6 **b**	49.3 ± 3.3 **b**	54.3 ± 4 **b**
	8	0 ± 0 **a**	0 ± 0 **b**	0 ± 0 **c**	1.8 ± 1.6 **c**	5 ± 2 **c**	9.6 ± 2.1 **c**	15 ± 2.6 **c**	22.5 ± 2.6 **c**	28.8 ± 2.4 **c**	34.4 ± 2.5 **c**	39.4 ± 2.7 **c**	42.7 ± 3 **c**
	12	0 ± 0 **a**	0 ± 0 **b**	0 ± 0 **c**	0 ± 0 **c**	1 ± 0.7 **d**	2.8 ± 1.1 **d**	4.9 ± 1.6 **d**	8 ± 2 **d**	13.2 ± 2.6 **d**	18.7 ± 3 **d**	22.9 ± 3.4 **d**	26 ± 4.2 **d**
AS	0	0 ± 0 **a**	1.9 ± 1.1 **a**	6.6 ± 1.6 **a**	12.8 ± 2.5 **a**	20.4 ± 3.4 **a**	31.5 ± 3.3 **a**	42.1 ± 2.9 **a**	50.6 ± 3.3 **a**	58.4 ± 3.9 **a**	64.5 ± 3.7 **a**	68.8 ± 4.1 **a**	71.8 ± 4.4 **a**
	4	0 ± 0 **a**	1.5 ± 1.5 **ab**	6 ± 2.2 **a**	11.1 ± 3.1 **a**	19.1 ± 4.4 **a**	26.4 ± 5.3**a**	33.2 ± 6.3 **b**	38.3 ± 6.5 **b**	42.6 ± 6.1 **b**	46.4 ± 5.6 **b**	49.8 ± 5.1 **b**	52.8 ± 4.6 **b**
	8	0 ± 0 **a**	0 ± 0 **b**	0 ± 0 **b**	0 ± 0 **b**	1.2 ± 1.1 **b**	5.8 ± 1.4 **b**	10.9 ± 1.9 **c**	16.6 ± 2.3 **c**	25.1 ± 1.7 **c**	33.1 ± 1.7 **c**	40.3 ± 2.1 **c**	46.5 ± 2.8 **b**
	12	0 ± 0 **a**	0 ± 0 **b**	0 ± 0 **b**	0 ± 0 **b**	0.6 ± 0.9 **b**	2.6 ± 1 **b**	5.3 ± 1.5 **c**	8.8 ± 1.9 **d**	15.3 ± 2.6 **d**	21.2 ± 1.7 **d**	26.1 ± 1.9 **d**	29.8 ± 2.6 **c**
EK	0	0 ± 0 **a**	1.5 ± 1.1 **a**	5.9 ± 2.1 **a**	12 ± 2.2 **a**	19.3 ± 2.8 **a**	30.9 ± 3.2 **a**	41.6 ± 3.3 **a**	48.8 ± 4 **a**	54.8 ± 3.9 **a**	60.1 ± 4.2 **a**	64.5 ± 5.5 **a**	66.2 ± 4.8 **a**
	4	0 ± 0 **a**	0 ± 0 **b**	0.6 ± 0.6 **b**	6.4 ± 0.7 **b**	13.5 ± 1.6 **b**	21.2 ± 2.7 **b**	30.8 ± 3 **b**	38.3 ± 3.9 **b**	44.1 ± 4 **b**	49.4 ± 3.9 **b**	54.1 ± 3.9 **b**	57 ± 3.6 **b**
	8	0 ± 0 **a**	0 ± 0 **b**	0 ± 0 **b**	0 ± 0 **c**	1.6 ± 0.6 **c**	7.2 ± 0.9 **c**	13.2 ± 1.7 **c**	20.2 ± 2.7 **c**	30.7 ± 3.3 **c**	38.7 ± 3.2 **c**	42.3 ± 3.6 **c**	45.4 ± 3.7 **c**
	12	0 ± 0 **a**	0 ± 0 **b**	0 ± 0 **b**	0 ± 0 **c**	0.7 ± 0.4 **c**	3.4 ± 0.3 **c**	6.4 ± 0.4 **d**	9.6 ± 0.5 **d**	13.4 ± 0.6 **d**	19.7 ± 1.4 **d**	25.6 ± 2.1 **d**	30.5 ± 2.9 **d**
ES	0	0 ± 0 **a**	1.4 ± 1.1 **a**	6.5 ± 1.6 **a**	13.9 ± 1.2 **a**	21.6 ± 1.7 **a**	30.6 ± 2.6 **a**	39.2 ± 2.9 **a**	46.8 ± 3.6 **a**	53.9 ± 4.1 **a**	60.3 ± 3.7 **a**	66.1 ± 4.1 **a**	71.2 ± 3.7 **a**
	4	0 ± 0 **a**	0.8 ± 0.7 **ab**	5.2 ± 0.7 **a**	10 ± 0.9 **b**	15.5 ± 1.3 **b**	21.8 ± 1.4 **b**	28.2 ± 1 **b**	33.8 ± 1.4 **b**	39 ± 1.7 **b**	43.9 ± 1.9 **b**	48.6 ± 2.1 **b**	52.9 ± 2.4 **b**
	8	0 ± 0 **a**	0 ± 0 **b**	0 ± 0 **b**	1 ± 0.9 **c**	2.8 ± 1.2 **c**	5.3 ± 2.1 **c**	9.7 ± 3.3 **c**	16.5 ± 3.3 **c**	27.1 ± 3.9 **c**	35.4 ± 5.1 **c**	39.8 ± 5.7 **c**	41.9 ± 5.8 **c**
	12	0 ± 0 **a**	0 ± 0 **b**	0 ± 0 **b**	0 ± 0 **c**	0.9 ± 0.6 **c**	2.9 ± 1.2 **c**	5.2 ± 2.1 **c**	8.3 ± 3.4 **d**	12.4 ± 4.6 **d**	16.9 ± 5.1 **d**	20.3 ± 5.2 **d**	23 ± 5.9 **d**
SB	0	0 ± 0 **a**	1.7 ± 1.1 **a**	5.3 ± 1.7 **a**	12.5 ± 0.9 **a**	23.2 ± 1.5 **a**	34.4 ± 2.5 **a**	45.6 ± 2.9 **a**	55.2 ± 3.4 **a**	62.2 ± 4.3 **a**	67.6 ± 3.6 **a**	72 ± 3.8 **a**	74.1 ± 4.8 **a**
	4	0 ± 0 **a**	0 ± 0 **b**	1.1 ± 1.4 **a**	6.2 ± 1.3 **b**	12 ± 1 **b**	18.9 ± 0.8 **b**	27.5 ± 1.1 **b**	34.6 ± 1.7 **b**	40.8 ± 2.2 **b**	46.3 ± 2.6 **b**	51.3 ± 2.6 **b**	56.1 ± 2.6 **b**
	8	0 ± 0 **a**	0 ± 0 **b**	0 ± 0 **b**	1.3 ± 1 **c**	4.1 ± 2.3 **c**	9.2 ± 3.6 **c**	14.8 ± 4.8 **c**	21.7 ± 6.6 **c**	31.3 ± 5.9 **c**	37.8 ± 5.7 **c**	42.8 ± 5.9 **c**	46.3 ± 5.9 **c**
	12	0 ± 0 **a**	0 ± 0 **b**	0 ± 0 **b**	0 ± 0 **c**	0.3 ± 0.4 **d**	4.8 ± 0.4 **d**	9.9 ± 0.5 **c**	15.3 ± 0.8 **c**	21.1 ± 1.1 **d**	26.8 ± 1.4 **d**	30.7 ± 1.4 **d**	33.2 ± 2.2 **d**
GR	0	0 ± 0 **a**	1.4 ± 0.9 **a**	4.4 ± 1.7 **a**	9.1 ± 2.4 **a**	15.4 ± 2.8 **a**	22.3 ± 3.6 **a**	30.5 ± 2.6 **a**	38.7 ± 2.5 **a**	46.3 ± 2.2 **a**	53.3 ± 2.3 **a**	58.8 ± 2.9 **a**	63.6 ± 2.8 **a**
	4	0 ± 0 **a**	0 ± 0 **b**	0 ± 0 **b**	0.3 ± 0.3 **b**	1.9 ± 0.3 **b**	6.8 ± 0.3 **b**	15.7 ± 1.1 **b**	23.5 ± 2.1 **b**	29.5 ± 2.2 **b**	34.9 ± 2.7 **b**	39.8 ± 2.9 **b**	44.3 ± 3 **b**
	8	0 ± 0 **a**	0 ± 0 **b**	0 ± 0 **b**	0 ± 0 **b**	0 ± 0 **b**	0.6 ± 0.5 **c**	3.5 ± 1.3 **c**	8.5 ± 2.4 **c**	15 ± 2.6 **c**	19.9 ± 1.8 **c**	23.5 ± 1.9 **c**	26.4 ± 2.3 **c**
	12	0 ± 0 **a**	0 ± 0 **b**	0 ± 0 **b**	0 ± 0 **b**	0 ± 0 **b**	0 ± 0 **c**	0 ± 0 **d**	0 ± 0 **d**	0.2 ± 0.2 **d**	4 ± 0.9 **d**	8.7 ± 1 **d**	14.2 ± 1.4 **d**

*AK= Abu Khalifah, AS= Abu Suwayr, EK= El Kasasen, ES= El Shark, SB= Serabeum, GR= Grafenreuth.

Cumulative hatched juveniles were expressed as a percentage of total number of juveniles.

Cumulative hatched juveniles (± standard deviation) in a column at each population followed by the same letter are not significantly different according to Tukey HSD test at $P \leq 0.05$.

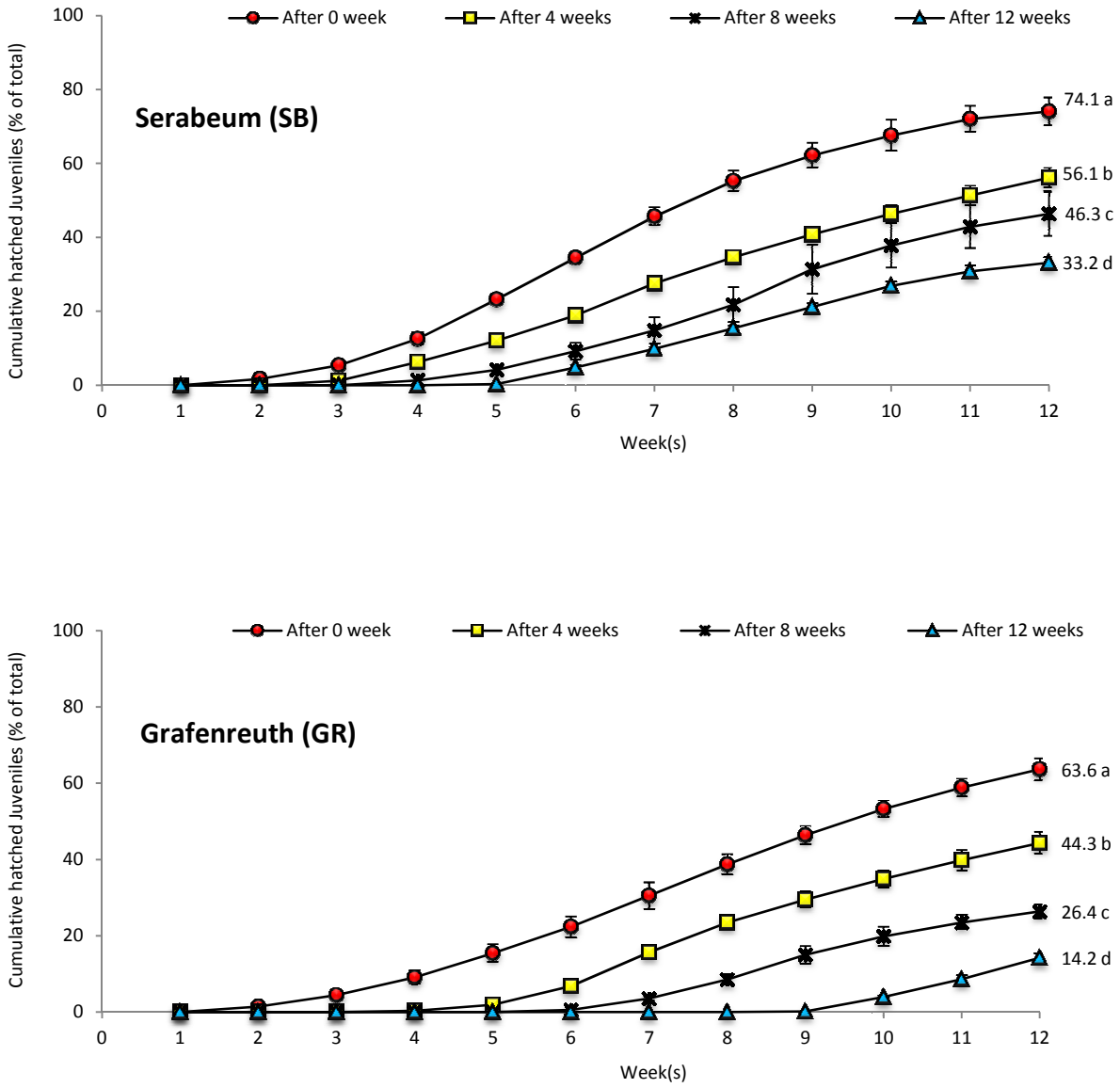

Figure 2. Influence of storage temperature at 30°C for different periods (0, 4, 8 and 12 weeks) prior incubation at 10°C over 12 weeks, on the cumulative hatch of one Egyptian population **(SB)** and one German population **(GR)** of *H. avenae*. Cumulative percentages of hatched juveniles followed by the same letter are not significantly different according to Tukey HSD test at $P \leq 0.05$. Vertical bars indicate standard deviation of the means.

The beginning of hatch of the tested populations after prior exposure of cysts to 30°C for 4 weeks was 2-5 weeks and the final cumulative hatch ranged between 44.3 - 57%. Hatching 4-7 weeks after prior exposure to 30°C for 8 weeks was observed and the tested populations showed final cumulative hatch between 26.4 - 46.5%. Juvenile emergence after 6-10 weeks was noticed after pre-treatment at 30°C for 12 weeks and the final cumulative hatch was the lowest compared with all other treatments and ranged from 14.2 to 33.2%.

Storing cysts at 20°C for 4, 8 or 12 weeks before incubation at 10°C for 12 weeks had different effects on the hatching behavior of the tested populations **(Table 4, Figure 3)**. Prior exposure of cysts to 20°C increased the hatching of all populations in comparison to the hatch from cysts that had not been stored at this temperature. Although, the storage period at 20°C resulted in a delay in hatching of the German population (after 3-4 weeks), rate of hatching was increased slightly (61.7-68.5%). These increases were positively correlated with the storage periods.

The Egyptian populations showed gradual increase in the hatching when stored at 20°C for 4, 8 or 12 weeks and then incubated at 10°C. The beginning of hatch of the Egyptian populations after prior exposure of cysts to 20°C for 4 weeks was in the second week and the hatching rate was ranged between 66.9 - 73.4%. Hatching in the first week after prior exposure to 20°C for 8 weeks was observed and the Egyptian populations showed final cumulative hatch between 77.8 – 84.7%. Juvenile emergence from the first week was noticed after storing the cysts at 20°C for 12 weeks and the final cumulative hatch was the highest compared to all other treatments and ranged from 89.5 – 94.7%.

Table 4. Influence of storage temperature at 20°C for different periods (0, 4, 8 and 12 weeks) prior incubation at 10°C over 12 weeks, on the cumulative hatch of *H. avenae* populations.

Pop.*	Storage period	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week12
AK	0	0 ± 0 **a**	2 ± 1.1 **a**	7.1 ± 1.6 **a**	14.4 ± 2.3 **a**	22.7 ± 3 **a**	31.5 ± 4 **a**	42.1 ± 3.7 **a**	50 ± 4 **b**	57.6 ± 3.7 **b**	64.2 ± 3.2 **b**	69.5 ± 3.2 **c**	74.2 ± 3.2 **c**
	4	0.2 ± 0.2 **a**	1.5 ± 0.9 **a**	7.8 ± 2.2 **a**	15.8 ± 3.1 **a**	24.6 ± 3.6 **a**	34.6 ± 4.5 **a**	45.9 ± 5.7 **a**	55 ± 6.2 **ab**	62.5 ± 6.3 **ab**	68 ± 5.7 **b**	71.4 ± 5 **bc**	73.4 ± 4.5 **c**
	8	0.9 ± 0.8 **a**	4.9 ± 1.1 **a**	10.5 ± 2.5 **a**	16.8 ± 3.6 **a**	24.6 ± 3.7 **a**	36.3 ± 5.1 **a**	46.8 ± 5.6 **a**	55.6 ± 5.6 **ab**	64.1 ± 5.4 **ab**	71.3 ± 4.5 **ab**	77.5 ± 3.4 **b**	82 ± 2.4 **b**
	12	0.9 ± 1 **a**	2.6 ± 1.5 **a**	7.1 ± 2.4 **a**	12.7 ± 3.5 **a**	18.8 ± 4.1 **a**	30.4 ± 4.2 **a**	45.9 ± 4.5 **a**	59.4 ± 4.3 **a**	70.2 ± 4.5 **a**	79.8 ± 5.2 **a**	86.9 ± 4.7 **a**	90.6 ± 3.7 **a**
AS	0	0 ± 0 **a**	1.9 ± 1.1 **ab**	6.6 ± 1.6 **a**	12.8 ± 2.5 **a**	20.4 ± 3.4 **ab**	31.5 ± 3.3 **ab**	42.1 ± 2.9 **b**	50.6 ± 3.3 **b**	58.4 ± 3.9 **b**	64.5 ± 3.7 **c**	68.8 ± 4.1 **c**	71.8 ± 4.4 **c**
	4	0.3± 0.4 **a**	1.4 ± 1.2 **b**	6.5 ± 2.3 **a**	13.5 ± 1.7 **a**	21.6 ± 1.8 **ab**	31.5 ± 1.5 **ab**	42.5 ± 1.4 **b**	51.3 ± 3 **b**	57 ± 2.8 **b**	61.9 ± 2 **c**	66.3 ± 1.2 **c**	68.6 ± 1.3 **c**
	8	0.7 ± 0.9 **a**	4.5 ± 2.2 **a**	9.5 ± 3.3 **a**	16.1 ± 3.1 **a**	24.6 ± 3 **a**	35.9 ± 4.6 **a**	53.5 ± 3.2 **a**	62.3 ± 2.6 **a**	70.3 ± 1.7 **a**	76.5 ± 1.6 **b**	81.1 ± 2.8 **b**	84.7 ± 3.5 **b**
	12	0.8 ± 0.6 **a**	3.3 ± 1.3 **ab**	7.7 ± 1.3 **a**	12.3 ± 1.3 **a**	17.3 ± 1.6 **b**	28.3 ± 1.8 **b**	44.1 ± 1.5 **b**	60.4 ± 1.9 **a**	72.7 ± 2.1 **a**	81.6 ± 1.8 **a**	87.6 ± 1.4 **a**	91.7 ± 1.8 **a**
EK	0	0 ± 0 **b**	1.5 ± 1.1 **a**	5.9 ± 2.1 **a**	12 ± 2.2 **a**	19.3 ± 2.8 **a**	30.9 ± 3.2 **a**	41.6 ± 3.3 **a**	48.8 ± 4 **bc**	54.8 ± 3.9 **bc**	60.1 ± 4.2 **c**	64.5 ± 5.5 **c**	66.2 ± 4.8 **c**
	4	0.2 ± 0.2 **b**	1.6 ± 1.6 **a**	7 ± 2.4 **a**	13.5 ± 3.7 **a**	21 ± 4.6 **a**	30.4 ± 5.9 **a**	39.7 ± 6.3 **a**	47.3 ± 5.6 **c**	53.9 ± 5 **c**	59.6 ± 3.8 **c**	64.1 ± 2.5 **c**	66.9 ± 2.2 **c**
	8	1.6 ± 0.8 **a**	4.1 ± 1.6 **a**	7.8 ± 2.2 **a**	12.6 ± 3.1 **a**	21.4 ± 4.5 **a**	32.3 ± 6.5 **a**	45.5 ± 5.5 **a**	56.5 ± 6 **ab**	65.8 ± 6.6 **ab**	73.8 ± 7.2 **b**	78.7 ± 6.6 **b**	82 ± 6.2 **b**
	12	0.9 ± 0.8 **ab**	2.4 ± 1.6 **a**	5.6 ± 2 **a**	10.9 ± 2.4 **a**	17.6 ± 2.8 **a**	30.9 ± 2.7 **a**	45.8 ± 2.9 **a**	63.5 ± 2.8 **a**	75.8 ± 2.7 **a**	85.8 ± 2.9 **a**	91 ± 1.8 **a**	94.7 ± 1.3 **a**
ES	0	0 ± 0 **a**	1.4 ± 1.1 **b**	6.5 ± 1.6 **b**	13.9 ± 1.2 **bc**	21.6 ± 1.7 **bc**	30.6 ± 2.6 **b**	39.2 ± 2.9 **b**	46.8 ± 3.6 **c**	53.9 ± 4.1 **c**	60.3 ± 3.7 **d**	66.1 ± 4.1 **c**	71.2 ± 3.7 **c**
	4	1 ± 0.8 **a**	3.3 ± 1.6 **ab**	9.2 ± 1.8 **ab**	16.2 ± 2 **ab**	25.5 ± 2.4 **ab**	36.1 ± 3.3 **ab**	45.1 ± 3.4 **b**	54 ± 4 **b**	61.6 ± 3.5 **b**	66.2 ± 2.5 **c**	69 ± 2.5 **c**	70.8 ± 2.8 **c**
	8	1 ± 0.8 **a**	5.3 ± 1.6 **a**	12.1 ± 2.1 **a**	19.3 ± 3 **a**	28.9 ± 4.2 **a**	42.8 ± 3.9 **a**	52.1 ± 4 **a**	60.9 ± 3.9 **a**	68.5 ± 3.2 **a**	73.8 ± 2.9 **b**	76.2 ± 3.7 **b**	77.8 ± 4.2 **b**
	12	0.9 ± 0.8 **a**	2.8 ± 1.9 **ab**	6.2 ± 2.2 **b**	11.7 ± 2.7 **c**	19.2 ± 3.5 **c**	29.6 ± 5 **b**	45.2 ± 2.9 **b**	59.1 ± 2.4 **ab**	71 ± 2.7 **a**	81.4 ± 3.4 **a**	86.9 ± 3 **a**	89.5 ± 2.9 **a**
SB	0	0 ± 0 **a**	1.7 ± 1.1 **b**	5.3 ± 1.7 **b**	12.5 ± 0.9 **b**	23.2 ± 1.5 **ab**	34.4 ± 2.5 **a**	45.6 ± 2.9 **a**	55.2 ± 3.4 **b**	62.2 ± 4.3 **b**	67.6 ± 3.6 **b**	72 ± 3.8 **b**	74.1 ± 4.8 **b**
	4	0.5 ± 0.6 **a**	1.7 ± 1.1 **b**	5.2 ± 2.3 **b**	12.4 ± 2.4 **b**	20.4 ± 2.9 **b**	31.4 ± 4.2 **a**	44.8 ± 6 **a**	54.3 ± 5.3 **b**	62.6 ± 4.3 **b**	68.6 ± 3 **b**	71.4 ± 3 **b**	73.1 ± 2.8 **b**
	8	0.8 ± 0.8 **a**	4.3 ± 2.3 **a**	11.2 ± 3 **a**	18.8 ± 3.7 **a**	27.4 ± 4.7 **a**	36.5 ± 5.7 **a**	46.5 ± 5.5 **a**	55.9 ± 5.3 **b**	64 ± 5.5 **b**	70.9 ± 5.3 **b**	77 ± 4.8 **b**	79.1 ± 4.2 **b**
	12	0.4 ± 0.5 **a**	1.9 ± 0.8 **ab**	5.4 ± 1.3 **b**	11 ± 1.4 **b**	20.2 ± 1.1 **b**	33.6 ± 3 **a**	49.8 ± 3.8 **a**	65.2 ± 3.9 **a**	76.9 ± 3.8 **a**	84.6 ± 3.1 **a**	91.2 ± 3 **a**	94 ± 2 **a**
GR	0	0 ± 0 **a**	1.4 ± 0.9 **a**	4.4 ± 1.7 **a**	9.1 ± 2.4 **a**	15.4 ± 2.8 **a**	22.3 ± 3.6 **a**	30.5 ± 2.6 **a**	38.7 ± 2.5 **a**	46.3 ± 2.2 **a**	53.3 ± 2.3 **a**	58.8 ± 2.9 **a**	63.6 ± 2.8 **a**
	4	0 ± 0 **a**	0.5 ± 0.5 **ab**	2.4 ± 0.9 **b**	7.7 ± 1.7 **a**	13.9 ± 2.1 **a**	20.8 ± 2.2 **ab**	31.2 ± 2.8 **a**	40.2 ± 3.6 **a**	47.8 ± 3.6 **a**	54.8 ± 3.8 **a**	59.9 ± 3.6 **a**	61.7 ± 3.5 **a**
	8	0 ± 0 **a**	0 ± 0 **b**	0.6 ± 0.4 **c**	2 ± 0.7 **b**	8.9 ± 2.1 **b**	16.6 ± 3.4 **bc**	27 ± 3.9 **a**	36.8 ± 4.6 **a**	45.6 ± 5.4 **a**	53.3 ± 6.5 **a**	59.9 ± 5.8 **a**	61.8 ± 5.9 **a**
	12	0 ± 0 **a**	0 ± 0 **b**	0 ± 0 **c**	0.8 ± 0.9 **b**	4 ± 2.7 **c**	14.7 ± 3.1 **c**	28.4 ± 2.4 **a**	40.5 ± 4.3 **a**	51.3 ± 6.3 **a**	59.6 ± 6.9 **a**	65.9 ± 6.7 **a**	68.5 ± 6.8 **a**

*AK= Abu Khalifah, AS= Abu Suwayr, EK= El Kasasen, ES= El Shark, SB= Serabeum, GR= Grafenreuth.

Cumulative hatched juveniles were expressed as a percentage of total number of juveniles.

Cumulative hatched juveniles (± standard deviation) in a column at each population followed by the same letter are not significantly different according to Tukey HSD test at $P \leq 0.05$.

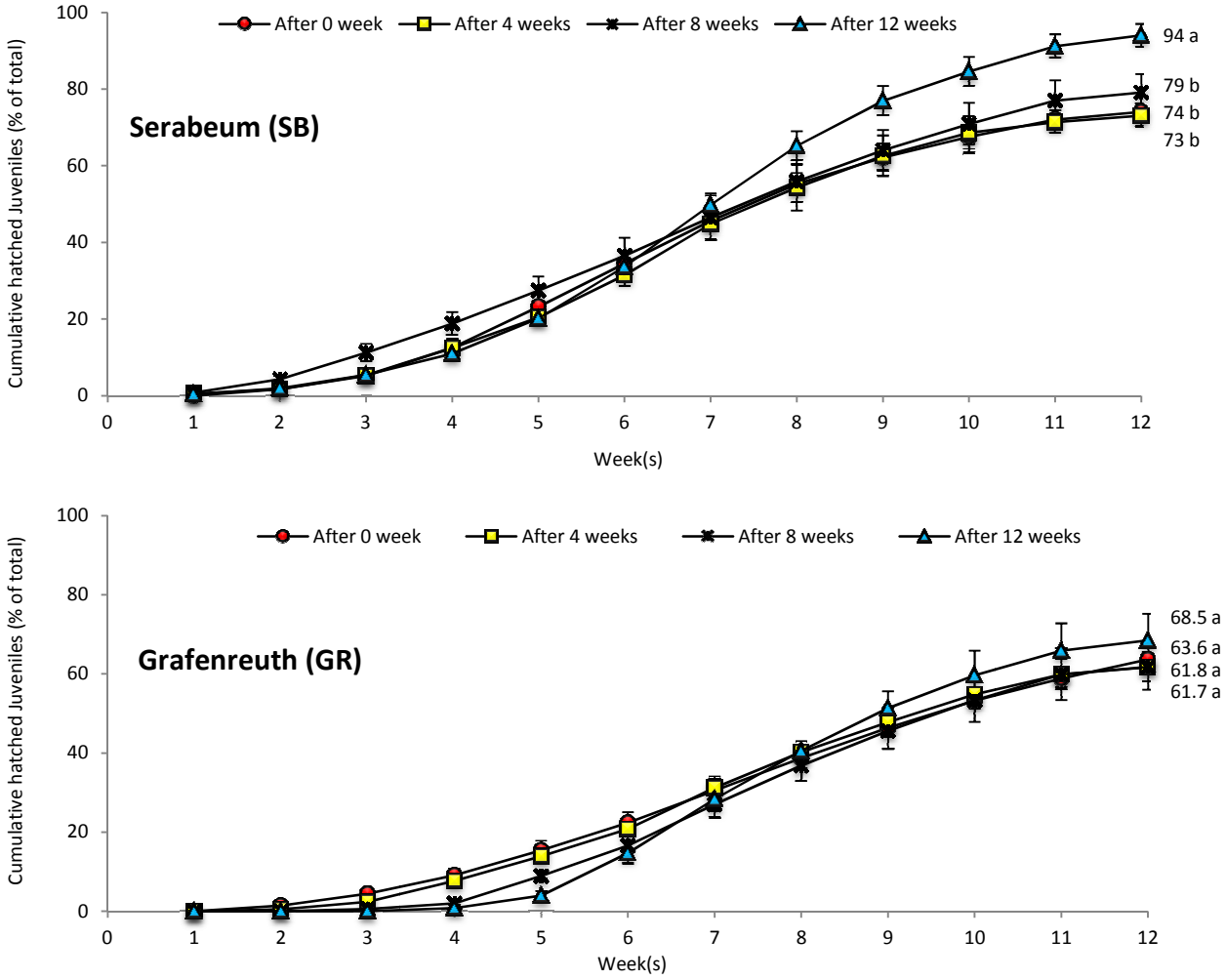

Figure 3. Influence of storage temperature at 20°C for different periods (0, 4, 8 and 12 weeks) prior incubation at 10°C over 12 weeks, on the cumulative hatch of one Egyptian population **(SB)** and one German population **(GR)** of *H. avenae*. Cumulative percentages of hatched juveniles followed by the same letter are not significantly different according to Tukey HSD test at $P \leq 0.05$. Vertical bars indicate standard deviation of the means.

Differences in hatching patterns between the German and Egyptian populations were noticed after storing the cysts at 5°C for 4, 8 or 12 weeks before incubation at 10°C for 12 weeks **(Table 5, Figure 4)**. The storage period of 5°C before incubation at 10°C resulted in a delay in hatching of the Egyptian populations, despite of the fact that the rate of hatching insignificantly increased. This induction was positively correlated with the storage period.

Table 5. Influence of storage temperature at 5°C for different periods (0, 4, 8 and 12 weeks) prior incubation at 10°C over 12 weeks, on the cumulative hatch of *H. avenae* populations.

Pop.*	Storage period	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week12
AK	0	0 ± 0 **a**	2 ± 1.1 **a**	7.1 ± 1.6 **a**	14.4 ± 2.3 **a**	22.7 ± 3 **a**	31.5 ± 4 **a**	42.1 ± 3.7 **a**	50 ± 4 **a**	57.6 ± 3.7 **ab**	64.2 ± 3.2 **b**	69.5 ± 3.2 **b**	74.2 ± 3.2 **ab**
	4	0 ± 0 **a**	1.6 ± 0.3 **ab**	5.9 ± 0.7 **a**	11.9 ± 1.2 **ab**	18.7 ± 2.1 **b**	29.1 ± 3 **a**	39.3 ± 3.2 **ab**	48 ± 3.5 **ab**	55.7 ± 4.4 **ab**	62.5 ± 4.8 **b**	67.4 ± 3.8 **b**	71.5 ± 3.1 **b**
	8	0 ± 0 **a**	0.9 ± 0.4 **bc**	5.2 ± 0.6 **a**	9.9 ± 1 **b**	16.1 ± 1.6 **ab**	23.4 ± 2.4 **b**	33.4 ± 2.5 **c**	42.9 ± 2.5 **b**	52.1 ± 2.6 **b**	61 ± 2.6 **ab**	67.6 ± 2.6 **b**	72.3 ± 2.6 **b**
	12	0 ± 0 **a**	0 ± 0 **c**	1.1 ± 1.2 **b**	3 ± 2.1 **c**	11 ± 2.9 **c**	20.6 ± 2.5 **b**	34.5 ± 2 **bc**	47.8 ± 1.5 **ab**	60.5 ± 1.1 **a**	70.9 ± 2.1 **a**	76.7 ± 2.6 **a**	79.2 ± 2.7 **a**
AS	0	0 ± 0 **a**	1.9 ± 1.1 **a**	6.6 ± 1.6 **a**	12.8 ± 2.5 **a**	20.4 ± 3.4 **a**	31.5 ± 3.3 **a**	42.1 ± 2.9 **a**	50.6 ± 3.3 **ab**	58.4 ± 3.9 **ab**	64.5 ± 3.7 **a**	68.8 ± 4.1 **a**	71.8 ± 4.4 **b**
	4	0± 0 **a**	1.7 ± 1.2 **a**	7.3 ± 2.6 **a**	14.3 ± 3.7 **a**	22.6 ± 4.5 **a**	31.8 ± 5.5 **a**	42.1 ± 5.5 **a**	50.8 ± 5.1 **ab**	58.9 ± 4.7 **ab**	66.4 ± 3.9 **a**	73.4 ± 3.7 **a**	75.5 ± 3.3 **ab**
	8	0 ± 0 **a**	1 ± 0.7 **ab**	6 ± 1.3 **a**	12.6 ± 2 **a**	20.5 ± 2 **a**	30 ± 1.9 **a**	41.8 ± 1.5 **a**	52.9 ± 0.9 **a**	63.3 ± 0.6 **a**	69 ± 1.3 **a**	73.3 ± 2.1 **a**	75.8 ± 2.1 **ab**
	12	0 ± 0 **a**	0 ± 0 **b**	0.9 ± 1.2 **b**	3.3 ± 2.4 **b**	8.6 ± 2.7 **b**	17.3 ± 2.8 **b**	30.6 ± 2.6 **b**	45.2 ± 2.5 **b**	57.3 ± 2.4 **b**	67.9 ± 2.2 **a**	74.7 ± 2.9 **a**	81.5 ± 3.7 **a**
EK	0	0 ± 0 **b**	1.5 ± 1.1 **a**	5.9 ± 2.1 **a**	12 ± 2.2 **a**	19.3 ± 2.8 **a**	30.9 ± 3.2 **a**	41.6 ± 3.3 **a**	48.8 ± 4 **a**	54.8 ± 3.9 **a**	60.1 ± 4.2 **a**	64.5 ± 5.5 **b**	66.2 ± 4.8 **b**
	4	0 ± 0 **b**	1.3 ± 1.1 **a**	4.4 ± 1.9 **a**	9.2 ± 2.8 **a**	16.6 ± 2.9 **a**	25.8 ± 1.4 **b**	36.5 ± 2.3 **ab**	45.9 ± 3 **a**	54 ± 3.2 **a**	60.1 ± 3.4 **a**	64.3 ± 3.7 **b**	67.4 ± 3.6 **b**
	8	0 ± 0 **a**	0.4 ± 0.6 **a**	4.8 ± 0.5 **a**	10 ± 0.8 **a**	16 ± 1.9 **a**	23.6 ± 2.4 **b**	34.2 ± 3 **b**	43.9 ± 3.7 **a**	52.8 ± 3.6 **a**	61.5 ± 3.6 **a**	68.1 ± 3.9 **ab**	72.9 ± 3.9 **ab**
	12	0 ± 0 **ab**	0 ± 0 **a**	0.4 ± 0.6 **b**	1.8 ± 1.5 **b**	6.9 ± 2.6 **b**	17.2 ± 2.4 **c**	32.4 ± 5.9 **b**	46.1 ± 6.4 **a**	57.6 ± 6.4 **a**	67.2 ± 6 **a**	73.2 ± 5.6 **a**	76.4 ± 5.7 **a**
ES	0	0 ± 0 **a**	1.4 ± 1.1 **ab**	6.5 ± 1.6 **a**	13.9 ± 1.2 **a**	21.6 ± 1.7 **ab**	30.6 ± 2.6 **ab**	39.2 ± 2.9 **a**	46.8 ± 3.6 **a**	53.9 ± 4.1 **a**	60.3 ± 3.7 **a**	66.1 ± 4.1 **a**	71.2 ± 3.7 **a**
	4	0 ± 0 **a**	1.4 ± 1.2 **ab**	4.4 ± 2.2 **a**	9.4 ± 3.4 **b**	17.2 ± 3.3 **b**	27.1 ± 3.1 **b**	38 ± 1.9 **a**	47.7 ± 2.3 **a**	56.6 ± 2.8 **a**	63.6 ± 2.7 **a**	68.1 ± 2.9 **a**	70.3 ± 4.3 **a**
	8	0 ± 0 **a**	1.9 ± 0.8 **a**	6.4 ± 2 **a**	14.4 ± 2.3 **a**	22.7 ± 3.2 **a**	32.7 ± 3.3 **a**	42.1 ± 4.5 **a**	50.5 ± 5.3 **a**	58.2 ± 6.4 **a**	63.1 ± 5.9 **a**	67.5 ± 4.7 **a**	70 ± 3.4 **a**
	12	0 ± 0 **a**	0 ± 0 **b**	0.3 ± 0.2 **b**	1.8 ± 1.6 **c**	8.4 ± 1.6 **c**	17.5 ± 2 **c**	32.2 ± 2.4 **b**	46.5 ± 2.8 **a**	57.8 ± 2.8 **a**	64.2 ± 2.7 **a**	69.9 ± 2.8 **a**	74.5 ± 3.4 **a**
SB	0	0 ± 0 **a**	1.7 ± 1.1 **a**	5.3 ± 1.7 **a**	12.5 ± 0.9 **a**	23.2 ± 1.5 **a**	34.4 ± 2.5 **a**	45.6 ± 2.9 **a**	55.2 ± 3.4 **a**	62.2 ± 4.3 **a**	67.6 ± 3.6 **a**	72 ± 3.8 **ab**	74.1 ± 4.8 **a**
	4	0 ± 0 **a**	1 ± 0.9 **ab**	3.9 ± 2 **a**	9.7 ± 2.2 **ab**	18.7 ± 2.4 **b**	31 ± 2.2 **a**	42.4 ± 2.3 **a**	52.4 ± 1.8 **a**	61.9 ± 1.8 **a**	70 ± 2.6 **a**	73.2 ± 2.3 **ab**	75.2 ± 2 **a**
	8	0 ± 0 **a**	0.8 ± 0.5 **ab**	3.5 ± 1.7 **a**	7.7 ± 2.2 **b**	12.4 ± 2.8 **c**	17.9 ± 3.5 **c**	29.4 ± 3.6 **c**	40.4 ± 4.2 **b**	50.1 ± 4.2 **b**	59.3 ± 4.2 **b**	68.1 ± 4.5 **b**	74 ± 4.7 **a**
	12	0 ± 0 **a**	0 ± 0 **b**	0.2 ± 0.2 **b**	1.2 ± 0.9 **c**	10.3 ± 0.9 **c**	22.8 ± 1.8 **b**	36.1 ± 2.7 **b**	51.5 ± 2.2 **a**	63 ± 1.6 **a**	71.1 ± 2.9 **a**	77.1 ± 3.5 **a**	80.8 ± 3.7 **a**
GR	0	0 ± 0 **b**	1.4 ± 0.9 **b**	4.4 ± 1.7 **b**	9.1 ± 2.4 **c**	15.4 ± 2.8 **c**	22.3 ± 3.6 **c**	30.5 ± 2.6 **c**	38.7 ± 2.5 **c**	46.3 ± 2.2 **c**	53.3 ± 2.3 **c**	58.8 ± 2.9 **c**	63.6 ± 2.8 **c**
	4	0.8 ± 0.8 **ab**	3.1 ± 1.5 **ab**	8.2 ± 1.6 **a**	14.5 ± 1.6 **ab**	21.7 ± 2.1 **ab**	31.4 ± 3.3 **ab**	40.4 ± 4.6 **b**	47.6 ± 5 **b**	53.8 ± 5.6 **b**	59.2 ± 5.7 **c**	64.4 ± 6 **c**	68.4 ± 6.9 **c**
	8	0.3 ± 0.3 **b**	2.4 ± 0.6 **ab**	6.4 ± 1 **ab**	11.2 ± 1.7 **bc**	17.6 ± 1.8 **bc**	25.9 ± 2.7 **bc**	38.9 ± 2.5 **b**	50.3 ± 2.5 **b**	59.7 ± 2.5 **b**	67.8 ± 1.6 **b**	73.8 ± 0.9 **b**	2.2 ± 5.9 **b**
	12	1.3 ± 0.8 **a**	3.6 ± 1.2 **a**	7.3 ± 1.1 **a**	15.3 ± 2.1 **a**	24.7 ± 2.9 **a**	35.5 ± 3.3 **a**	49.3 ± 3.9 **a**	60.4 ± 3.8 **a**	69.8 ± 4 **a**	77.2 ± 3.3 **a**	84 ± 3.2 **a**	90.1 ± 3.3 **a**

*AK= Abu Khalifah, AS= Abu Suwayr, EK= El Kasasen, ES= El Shark, SB= Serabeum, GR= Grafenreuth.

Cumulative hatched juveniles were expressed as a percentage of total number of juveniles.

Cumulative hatched juveniles (± standard deviation) in a column at each population followed by the same letter are not significantly different according to Tukey HSD test at $P \leq 0.05$.

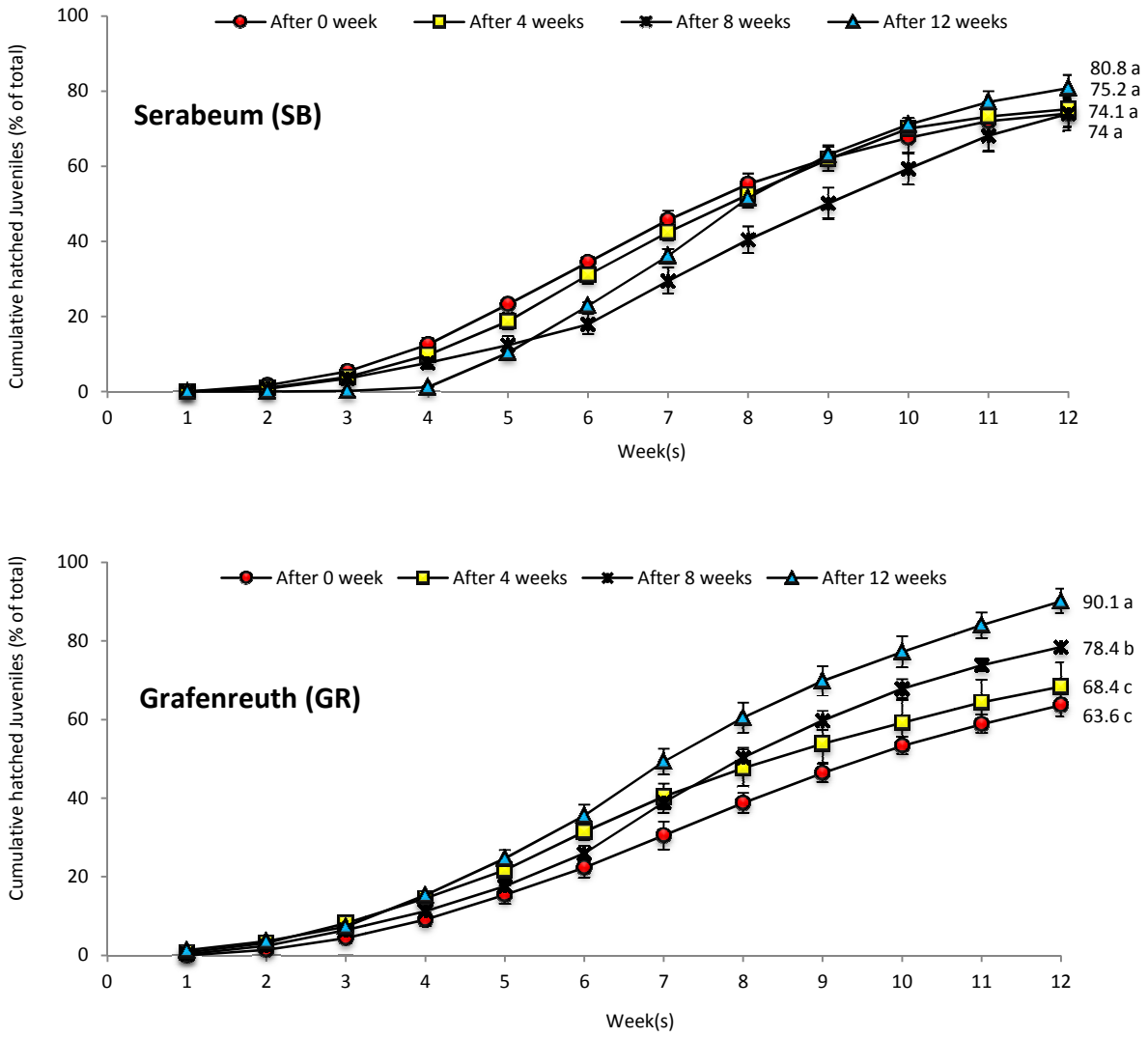

Figure 4. Influence of storage temperature at 5°C for different periods (0, 4, 8 and 12 weeks) prior incubation at 10°C over 12 weeks, on the cumulative hatch of one Egyptian population **(SB)** and one German population **(GR)** of *H. avenae*. Cumulative percentages of hatched juveniles followed by the same letter are not significantly different according to Tukey HSD test at $P \leq 0.05$. Vertical bars indicate standard deviation of the means.

Juvenile emergence from the Egyptian populations was recorded from the second week after storage at 5°C for 4 weeks and the final cumulative hatch ranged between 67.4 – 75.5%. Hatching after 2-3 weeks following prior exposure to 5°C for 8 weeks was observed with the Egyptian populations which showed final cumulative hatch between 70 – 75.8%. Moreover, the beginning of hatch of the Egyptian populations following

prior exposure of cysts to 5°C for 12 weeks was after 3-4 weeks and the hatching rate was ranged between 74.5 – 81.5%.

On the other hand, a significant stimulation in hatch (68.4%) of the German population was obvious after 4 weeks storage at 5°C. After 8 weeks storage, there was a significant stimulatory effect in the hatching of the German population (78.4%). However, the earliest (from the first week) and the highest final cumulative hatch (90.1%) compared to all other treatments was observed after storing the cysts at 5°C for 12 weeks before incubation at 10°C for 12 weeks.

DISCUSSION

Hatching experiments with the cereal cyst nematode *H. avenae* from distinct geographic areas of Europe, Australia and North America have shown clear variation in the hatching patterns of *H. avenae* **(Fushey and Johnson, 1966; Banyer and Fischer, 1971b; Rivoal, 1979, 1986; Zancada and Sanchez, 1988; Rivoal and Ireholm, 1990)**. These patterns correspond to two major factors or ecotypes, distinguished by activity in either winter (Mediterranean ecotype) or spring (Northern ecotype from oceanic and more or less temperate climates). These ecotypes appear to represent an adaptation to climate that synchronizes parasite hatch with the most favorable conditions for infection of the plant host and for survival of the nematode **(Rivoal, 1982)**.

Germany has a temperate climate and the season for growing wheat is long, generally 10 to 11 months, beginning in October and ending in July. The optimum temperature for hatch of *H. avenae* from Germany (Grafenreuth) was between 5 and 10°C. These temperatures usually occur in wheat fields of Germany at spring time when the mean temperatures range between 3 to 14°C **(Figure 5-A)**. The hatching pattern of the German population was of the Northern type with spring activity and show similarity to *H. avenae* populations of North Spain **(Zancada and Sanchez, 1988)**; England **(Williams and Beane, 1979)**; and North Australia.

Experimental exposure of CCN to different temperatures to simulate seasonal variations was useful to explain the hatching processes of cereal cyst nematodes **(Rivoal, 1983; Mokabli *et al.,* 2001)**. Storing cysts at low temperatures before the hatching test were used to simulate the normal variation of soil temperature during the winter in Germany. Storage *H. avenae* cysts at 5°C for 8 or 12 weeks before incubation at 10°C stimulated the hatch of the German population significantly compared to the hatch from cysts that had not been stored at this temperature. This may explain that changes from winter (5°C pre-treatment) to spring (10°C incubation) warmer periods, cause rapid and brief hatch of juveniles. Stimulation of hatch of *H. avenae* populations after an exposure to 7°C was recorded by **Fushtey and Johnson (1966)** and **Banyer**

and Fisher (1971 a,b). **Mokabli *et al.,* (2001)** reported that exposure to 3°C for 2 months stimulated the hatch of *H. avenae* populations from Northern France. **Grabbert and Berger (1987)** reported an increase in hatching of *H. avenae* in Germany following exposure to 5°C for 16 days.

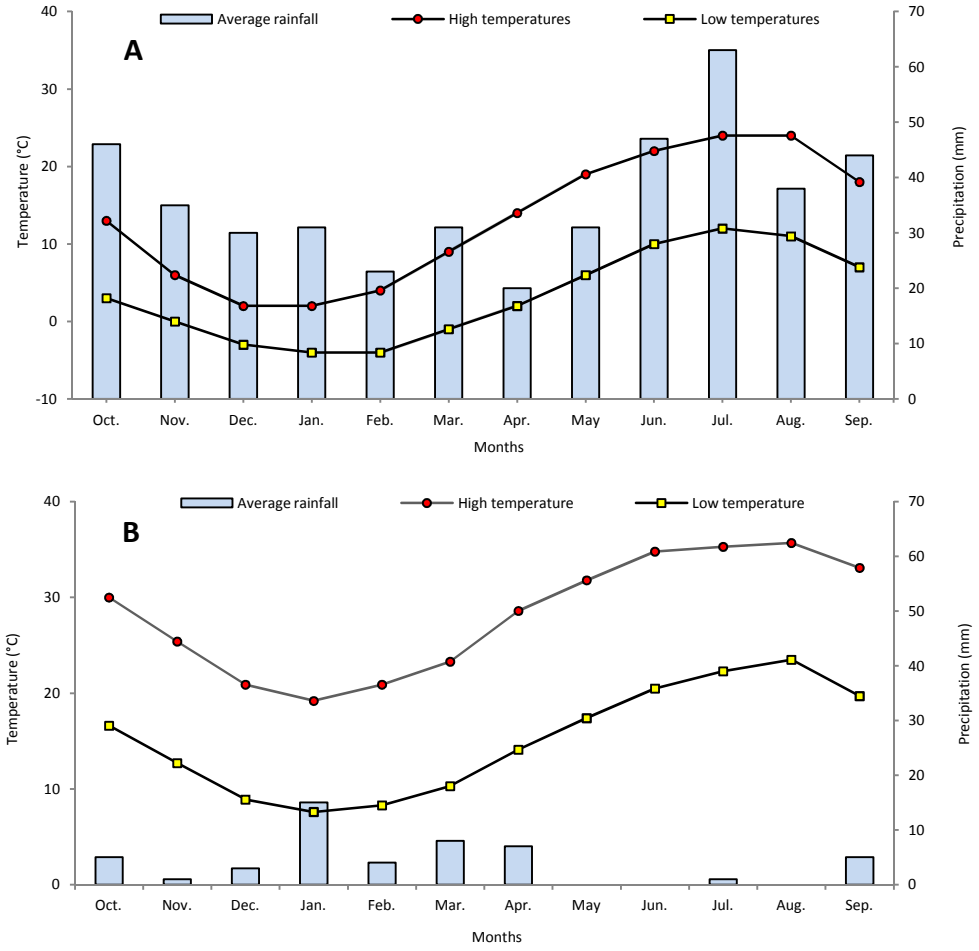

Figure 5. Monthly mean temperatures (high and low) and precipitation values in **A)** Grafenreuth, Bavaria, Germany; **B)** Ismailia province, Egypt.

Ismailia province of Egypt has a sub-tropical semi-arid Mediterranean climate. The winters are sufficiently temperate for wheat cultivations while summers are completely dry and warm, rainfall is negligible. Wheat planting begins around the first of October and ends at the end of November. Harvest periods begin around the first of May to the end of June. No significant differences in the hatching pattern were observed among the Egyptian populations at different temperature treatments. It is possible that

temperature and other agro-ecological factors in the surveyed regions of Ismailia might not be distinctly different as they were in France **(Rivoal, 1978)** or in Spain **(Zancada and Sanchez, 1988)**, where different hatching patterns of *H. avenae* related to the geographical origin of the populations were detected. The optimum temperature for hatch of the Egyptian populations was between 10 and 15°C. At these temperatures, the Egyptian *H. avenae* cysts showed the highest hatching rate and these temperatures usually occur in wheat fields of Ismailia province at winter time when the mean temperatures range between 8 to 20°C **(Figure 5-B)**. This hatching pattern of the Egyptian populations of *H. avenae* is similar to the Mediterranean ecotypes which have winter activity, such as *H. avenae* populations from Southern France **(Rivoal, 1978)**, Italy **(Greco, 1981)**, the drier areas of Spain **(Valdeolivas and Romero, 1986)**, Southern Australia **(Banyer and Fischer, 1971b)** and Israel **(Mor *et al.,* 1992)**.

The stimulation in the hatching of the Egyptian populations after exposing the cysts to 5°C for 8 or 12 weeks was not significantly different than the hatch from cysts that had not been exposed to this temperature. The same was observed with a population of *H. avenae* from Southern Italy, which does not require a cold stimulus to initiate hatching of eggs in new cysts **(Greco, 1981)**. On the other hand, storing cysts at high temperatures before the hatching test were used to simulate the normal variation of soil temperature during the summer. Exposing cysts to 30°C for 4, 8 or 12 weeks inhibited and delayed the hatch of all populations of *H. avenae* after incubating the cysts at 10°C. This indicates that a temperature of 30°C is sufficiently high to inhibit hatching, possibly do considerable injury to the contents of the cysts or may induce dormancy.

Storing cysts at moderate temperatures before the hatching test were used to simulate the normal variation of soil temperature during the autumn in Egypt. Exposing cysts to 20°C for 4, 8 or 12 weeks stimulated the hatch of the Egyptian populations of *H. avenae* significantly after incubating the cysts at 10°C. This highest level of emergence obtained by the Egyptian populations in the series moved from 20° to 10° corresponds with the observation of **Fisher (1981)** on an Australian population. Likewise, most *H. avenae* juveniles of the Spanish populations, emerged from cysts transferred from 20° to 10°, with 77-95% **(Valdeolivas and Romero, 1986)**.

In Egypt, cysts of *H. avenae* usually mature on host roots in late spring and in the soil in early summer, and then eggs inside the cysts have to survive the dry summer. When temperatures drop below 30°C after summer to about 20°C in autumn and about 10°C in winter, hatching may start again. If the temperature is adequate for a sufficiently long period almost all juveniles leave the cyst and a mass invasion of young cereal roots in late autumn or in winter may occur, suggesting a substantial hatch at this time in the field **(Banyer and Fischer, 1971b)**. The same hatching behavior was noticed in Italy, where cereals are sown in autumn, after dry and hot summer conditions which are unsuitable for nematodes **(Greco, 1981)**. Similar behavior was noticed in Israel by **Mor *et al.,* (1992)**; with the onset of autumn, juveniles began to emerge from cysts and attacked roots of germinating wheat seedlings. Hatching and root penetration continued through the winter. In spring, numbers of mature females on roots increased. Cysts oversummered in the soil until the following wheat season.

The results from this study may be useful for the development of control strategies to *H. avenae*. Synchronization between hatching of *H. avenae* and the sowing period of wheat under Mediterranean conditions is likely to result in heavy early infestation and crop losses. Adjustment of sowing dates to escape synchrony of peak emergence with the more sensitive stage of the crop could maximize the final yield. Control strategies such as early planting, minimum tillage and rotation that are effective against the Mediterranean ecotype of *H. avenae* in southern France and Spain **(Romero *et al.,* 1991; Rivoal and Cook, 1993)** could be applied or developed against the Egyptian populations of *H. avenae*.

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CHAPTER 4

Virulence characterization of cereal cyst nematode populations (*Heterodera avenae* Wollenweber) from Egypt and host responses of wheat cultivars

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ABSTRACT

The cereal cyst nematode (CCN), *Heterodera avenae* Wollenweber, causes serious economic losses in cereal crops. The use of resistant germplasm to control CCN is considered cost effective and environmentally friendly. Several pathotypes of *H. avenae* have been reported. The use and effectiveness of resistant wheat cultivars varies according to the virulence phenotype of the nematode population. *Heterodera avenae* has been reported in wheat fields in Egypt. As yet there is no information available on the virulence and damage potential of these populations. In this study, *H. avenae* populations from Egypt were characterized on a set of differential test cultivars and local Egyptian wheat varieties. The Egyptian populations belong to the same virulence phenotype and were virulent on all tested wheat cultivars. The Egyptian populations and the German population belong to different pathotypes. The Egyptian populations expressed virulence similar to that of pathotype Ha13. The German population could be assigned to pathotype Ha11. The Egyptian local cultivar 'Sakha 93' was the only wheat cultivar that is tolerant to all *H. avenae* populations tested. The reduction of grain yield of 'Sakha 93' by all tested nematode populations was not significantly reduced in spite of its high relative susceptibility to *H. avenae*.

Keywords - *Heterodera avenae*, wheat, pathotype, virulence, resistance, tolerance, grain yield.

INTRODUCTION

The cereal cyst nematode (CCN) *Heterodera avenae* Wollenweber, causes serious economic losses in cereal crops. In Egypt, *H. avenae* has been reported in wheat fields for the first time by **Ibrahim *et al.,* 1986** on barley and wheat in the Nile Delta and other localities of Northern Egypt. In **2007, Ibrahim and Handoo** reported the occurrence of *H. avenae* on Egyptian wheat in Alexandria and El-Behera governorates. Recently, *H. avenae* were found in wheat fields of Abu Khalifah, Abu Suwayr, El Kasasen, El Shark (West Sinai), and Serabeum regions in Ismailia province **(chapter 2)**.

Integrated pest management options have been developed for cereal cyst nematode, involving rotation with non-hosts and the use of resistant germplasm is considered an effective method of reducing CCN populations **(Nicol and Rivoal, 2008)**. One of the major obstacles and challenges to using host plant resistance is to determine pathotypes/virulent populations.

A pathotype scheme to distinguish CCN populations and pathotypes using an International Test Assortment of barley, oat and wheat cultivars was developed by several authors **(Andersen and Andersen, 1982b; Cook and Noel, 2002; Cook and Rivoal, 1998; Kretschmer *et al.,* 1997; Nicol, 2002; Persondedryver and Doussinault, 1984; Rivoal and Cook, 1993; Sanchez and Zancada, 1987; Smiley *et al.,* 2011)**. The pathotype scheme distinguishes three groups of pathotypes of *H. avenae* by differential resistance or susceptibility reactions **(Nicol and Rivoal 2007)**. Groups 1 and 2 include the largest number of pathotypes that occur in Europe, North Africa, and Asia **(Al-Hazmi *et al.,* 2001; Andersen and Andersen 1982a; Mokabli *et al.,* 2002)**. Pathotypes within Group 3 have been identified mostly in Australia and Europe **(Nicol and Rivoal 2007)**. Characterization of the pathotype(s) of *H. avenae* at each location is required to employ or develop resistant cultivars that can be used in integrated management systems to control these nematodes **(Al Hazmi *et al.,* 2001)**.

Heterodera avenae populations from Egypt were characterized for their virulence against a number of discriminating wheat cultivars. In this study resistance and tolerance of some local Egyptian wheat cultivars to *H. avenae* populations were investigated. The generated information will assist in choosing resistant cultivars for the development of control strategies against cereal cyst nematode *H. avenae* in Egypt.

MATERIALS AND METHODS

Nematode populations

Six *H. avenae* populations **(Table 1)** were characterized for their virulence against differential wheat cultivars. Five Egyptian populations were collected from different wheat growing areas in Ismailia province, and one German population (originally from Grafenreuth, Bavaria) were used in this experiment. Nematode cysts were dried at room temperature (15±2°C) and kept at 7°C until further use. Nematodes were reared on the Egyptian wheat (*Triticum aestivum*) cultivar 'Sakha 93' and newly formed cysts were used in the experiment. Cysts were squashed according to **Seinhorst and Den Ouden (1966)** and the total numbers of eggs and second stage juveniles (J2) per cyst were counted to determine cyst contents.

Table 1. Origin of *H. avenae* populations used in this study.

Code	Location	Country	Cyst contents	No. of cysts used per pot
AK	Abu Khalifah region, Ismailia	Egypt	76.3±3.7	35
AS	Abu Suwayr region, Ismailia	Egypt	78.7±3.2	34
EK	El Kasasen region, Ismailia	Egypt	87.2±4.9	31
ES	El Shark region (West Sinai), Ismailia	Egypt	87.0±2.8	31
SB	Serabeum region, Ismailia	Egypt	84.0±5.2	32
HA	Grafenreuth, Bavaria	Germany	81.3±5.0	33

Plant material

Fourteen wheat cultivars **(Table 2)** were used in this study. Five standard wheat cultivars from the International Test Assortment (NordGen, Alnarp, Sweden) for cereal cyst nematode pathotypes determination were used to characterize the nematode populations.

Nine locally-grown bread-wheat cultivars from Egypt were screened for resistance and tolerance against *H. avenae* populations.

Table 2. Origin of wheat (*Triticum aestivum*) cultivars used to characterize populations of *H. avenae*.

Entry	Code	Accession no.	Origin
Aus 10894*	AUS	NGB11099	Denmark
Capa*	CAP	NGB4823	--
Gemmeza 7**	G7	--	Egypt
Gemmeza 9**	G9	--	Egypt
Giza 168**	GIZA	--	Egypt
Iskamish K-2-Light*	ISK	NGB11091	Afghanistan
Loros X Koga (63/1.7.15.12)*	LOR	NGB11090	Denmark
Psathias*	PSA	NGB11098	Australia
Sahl 1**	SAHL	--	Egypt
Sakha 61**	S61	--	Egypt
Sakha 8**	S8	--	Egypt
Sakha 93**	S93	--	Egypt
Sakha 95**	S95	--	Egypt
Seds 1**	SEDS	--	Egypt

* Wheat cultivars from International Test Assortment, NordGen, Alnarp, Sweden.
**Local Egyptian cultivars from Department of Crops, Suez Canal University, Ismailia, Egypt.

Experimental set-up

Plastic pots (500ml) were filled with a sterilized soil mixture (2 loam: 1 field soil), fertilized with a granular fertilizer (Osmocote Exact Standard® 1.5g/kg soil). Cysts of *H. avenae* populations were added to the pots to give initial population densities of 5 juveniles per ml soil (see **table 1** for number of cysts added). Five pots were used as replicates for each nematode population. Each pot contained five seedlings of the respective wheat cultivar **(Table 2)**. For each cultivar, five pots of non-infested soil served as control. Plants were grown in a greenhouse at 20±3°C (16 h light/8 h dark) and watered as necessary with tap water.

Data collection and analysis

After 4 months, the final nematode numbers (P_f) were determined. Cysts were extracted from the soil using the floatation technique **(Shepherd, 1986)**. Counting and

separation of cysts from soil debris and other organic materials retained on the filter paper were carried out at 25x magnification under a stereoscopic binocular (Leica MZ8). Cysts were squashed according to **Seinhorst and Den Ouden (1966)** and the number of eggs and juveniles were counted.

Resistance was assessed as relative susceptibility (*RS*) to the standard susceptible control cultivar 'Capa' (*RS*= P_f on the test cultivar/P_f on susceptible control 'Capa')*100, where *Pf*= final population density of eggs and J2/ml soil. A rating system based on the relative susceptibility was used to characterize the host response of different wheat cultivars **(Lücke, 1976)**. Cultivars with *RS* less than 5% were considered resistant. The cultivars were considered moderately resistant if *RS* was between 6-20%. Cultivars with 21-50% relative susceptibility were considered moderately susceptible while cultivars with *RS* > 51% were recorded as susceptible cultivars **(Lücke, 1976).**

To characterize the pathotypes, responses of the tested differentials were compared with an updated matrix given by **Smiley *et al.* (2011)**.

Growth parameters (shoot dry weight, root dry weight, spike weight and grain yield per pot) were recorded to determine the damage potential of *H. avenae* populations on wheat cultivars. The percentages of reduction in different plant growth parameters were calculated as follows: *Red* (%) = ((*CP-IP*)/*CP*)*100, where *Red* (%) = percentage of reduction, *CP*= growth parameters of control plant, *IP*= growth parameters of infested plant. The tolerance index of a cultivar at each nematode initial population density was calculated as follows: *TI*= ((*GCP-GIP*)/*GCP*)*100/P_f, where *TI*= tolerance index, *GCP*= grain yield of control plant, *GIP*= grain yield of infested plant, P_f= final population density of eggs and J2/ml soil. Wheat cultivars with *TI* less than 0.5 considered tolerant. Wheat cultivars were less tolerant when *TI* was between 0.5 – 1. Wheat cultivars with *TI* higher than 1, were considered sensitive to *H. avenae* populations **(Dixon *et al.,* 1990)**.

Levene's test was used to test homogeneity of variances. Data were analyzed using ANOVA (SPSS version 19.0, IBM Corporation, New Orchard Road Armonk, New York, United States). Means were separated using Tukey HSD test at $P \leq 0.05$.

RESULTS

Virulence of *H. avenae* populations to wheat cultivars

The Egyptian populations of *H. avenae* showed similar virulence characteristics on the differentials of the International Test Assortment and on the local wheat cultivars **(Table 3)**. All the Egyptian populations were virulent to the tested wheat cultivars. The German population was avirulent and did not reproduce on the wheat lines 'Loros x Koga' and 'Aus 10894', while this population was virulent to all other cultivars ('Capa', 'G7', 'G9', 'Giza 168', 'Iskamish', 'Psathias', 'Sakha 61', 'Sakha 8', 'Sakha 93' and 'Sakha 95').

Resistance of wheat cultivars to *H. avenae* populations

Final population densities of *H. avenae* populations and relative susceptibility of different wheat cultivars are presented in **Table 3**. Few differences between the Egyptian populations, in resistance rating with the local wheat cultivars cv. 'Gemmeza 7' and 'Gemmeza 9' were recorded. These two cultivars were moderate susceptible to the nematode populations ES and SB and showed relative susceptibility (*RS*) between 38.4 – 40.4 and 38 – 40.1%, respectively. They were susceptible to the Egyptian populations AK, AS and EK and showed *RS* between 53.6 – 55.4 and 51 – 53.1%, respectively.

The wheat cv. 'Loros x Koga' and 'Aus 10894' were moderately resistant to the Egyptian populations and their relative susceptibility was between 11.1 – 19.6 and 13.7 – 19.9%, respectively. Amongst the local tested cultivars, 'Sahl 1' and 'Seds 1' were moderate susceptible to the Egyptian populations and showed the lowest *RS* which ranged between 20.7 – 26.6 and 20.6 – 27.7%, respectively. The wheat cultivars 'Iskamish', 'Psathias', 'Sakha 61', 'Sakha 8', 'Sakha 93' and 'Sakha 95' proved to be susceptible to the Egyptian populations of *H. avenae*. The highest susceptibility was recorded for cultivars 'Sakha 8' and 'Sakha 93' which showed *RS* between 94.5 – 114.3 and 97.2 – 117.6%, respectively.

Table 3. Final population densities of *H. avenae* populations and relative susceptibility of different wheat cultivars.

Pop. / Cult.	Abu Khalifah			Abu Suwayr			El Kasasen			El Shark			Serabeum			Grafenreuth		
	P_f [a]	*RS* [b]	*Rank* [c]	P_f	*RS*	*Rank*	P_f	*RS*	*Rank*	P_f	*RS*	*Rank*	P_f	*RS*	*Rank*	P_f	*RS*	*Rank*
Aus 10894	10.6 ± 1.1 **ab**	17.5	**(R)**	9.60 ± 1.3 **b**	16.9	**(R)**	12.7 ± 1.7 **a**	19.9	**(R)**	10.0 ± 1.2 **b**	14.8	**(R)**	8.40 ± 0.5 **b**	13.7	**(R)**	2.60 ± 0.4 **c**	4.5	**R**
Capa	60.6 ± 4.4 **ab**	100	**S**	56.7 ± 6.1 **b**	100	**S**	63.7 ± 6.1 **ab**	100	**S**	67.5 ± 5.8 **a**	100	**S**	61.3 ± 4.3 **ab**	100	**S**	57.9 ± 6.6 **ab**	100	**S**
Gemmeza 7	33.0 ± 2.3 **ab**	54.5	**S**	30.4 ± 3.6 **abc**	53.6	**S**	35.3 ± 1.3 **a**	55.4	**S**	27.3 ± 2.0 **cd**	40.4	**(S)**	24.6 ± 2.3 **d**	40.1	**(S)**	30.2 ± 2.8 **bc**	52.2	**S**
Gemmeza 9	31.5 ± 3.7 **ab**	52.0	**S**	30.1 ± 4.3 **ab**	53.1	**S**	32.5 ± 1.3 **a**	51.0	**S**	25.9 ± 1.4 **bc**	38.4	**(S)**	23.3 ± 2.3 **bc**	38	**(S)**	26.7 ± 3.9 **bc**	46.1	**(S)**
Giza 168	37.7 ± 5.3 **ab**	62.2	**S**	31.4 ± 5.3 **b**	55.4	**S**	42.4 ± 3.9 **a**	66.6	**S**	44.7 ± 4.2 **a**	66.2	**S**	37.6 ± 4.4 **ab**	61.3	**S**	35.8 ± 3.6 **ab**	61.8	**S**
Iskamish K-2	31.7 ± 2.4 **a**	52.3	**S**	31.8 ± 2.7 **a**	56.1	**S**	35.4 ± 1.6 **a**	55.6	**S**	35.2 ± 2.6 **a**	52.1	**S**	31.2 ± 2.4 **a**	50.9	**S**	33.3 ± 2.4 **a**	57.5	**S**
Loros x Koga	9.20 ± 1.3 **ab**	15.2	**(R)**	11.1 ± 1.5 **a**	19.6	**(R)**	11.1 ± 0.7 **a**	17.4	**(R)**	7.50 ± 1.2 **bc**	11.1	**(R)**	6.80 ± 0.5 **c**	11.1	**(R)**	2.40 ± 0.4 **d**	4.1	**R**
Psathias	33.3 ± 3.6 **a**	55.0	**S**	35.5 ± 2.4 **a**	62.6	**S**	33.0 ± 2.3 **a**	51.8	**S**	35.6 ± 3.3 **a**	52.7	**S**	32.7 ± 3.5 **a**	53.3	**S**	36.1 ± 3.8 **a**	62.3	**S**
Sahl 1	14.6 ± 2.1 **a**	24.1	**(S)**	15.1 ± 3.6 **a**	26.6	**(S)**	16.3 ± 2.0 **a**	25.6	**(S)**	14.7 ± 0.9 **a**	21.8	**(S)**	12.7 ± 1.9 **a**	20.7	**(S)**	14.1 ± 2.1 **a**	24.4	**(S)**
Sakha 61	47.3 ± 6.9 **bc**	78.1	**S**	45.8 ± 6.7 **c**	80.8	**S**	57.5 ± 5.3 **ab**	90.3	**S**	59.6 ± 3.6 **a**	88.3	**S**	52.8 ± 4.6 **abc**	86.1	**S**	43.9 ± 6.2 **c**	75.8	**S**
Sakha 8	62.1 ± 3.5 **bc**	102.5	**S**	53.6 ± 6.4 **c**	94.5	**S**	72.8 ± 3.2 **ab**	114.3	**S**	76.0 ± 3.1 **a**	112.6	**S**	64.5 ± 6.7 **bc**	105.2	**S**	41.7 ± 8.8 **d**	72	**S**
Sakha 93	67.4 ± 4.4 **ab**	111.2	**S**	57.6 ± 6.3 **bc**	101.6	**S**	74.9 ± 2.5 **a**	117.6	**S**	71.7 ± 3.9 **a**	106.2	**S**	59.6 ± 7.7 **bc**	97.2	**S**	54.9 ± 6.4 **c**	94.8	**S**
Sakha 95	49.1 ± 4.5 **ab**	81.0	**S**	42.4 ± 3.2 **b**	74.8	**S**	53.5 ± 4.1 **a**	84.0	**S**	54.9 ± 3.2 **a**	81.3	**S**	51.3 ± 3.4 **a**	83.7	**S**	48.2 ± 4.6 **ab**	83.2	**S**
Seds 1	16.1 ± 3.5 **a**	26.6	**(S)**	15.7 ± 3.4 **a**	27.7	**(S)**	13.1 ± 1.2 **a**	20.6	**(S)**	15.0 ± 1.3 **a**	22.2	**(S)**	13.7 ± 2.0 **a**	22.3	**(S)**	14.8 ± 2.5 **a**	25.6	**(S)**

[a] P_f = final population density of eggs and J2/ml soil ± standard deviation. P_f means in a row followed by the same letter are not significantly different based on Tukey test ($P \leq 0.05$).

[b] *RS* (Relative susceptibility %) = P_f on the test cultivar/P_f on susceptible control 'Capa')*100.

[c] *Rank* (Resistance ranking) according to Lücke (1976): R, resistant (0-5%); (R), moderately resistant (6-20%); (S), moderately susceptible (21-50%); and S, susceptible (>51%).

The German population of *H. avenae* could be differentiated from the Egyptian populations by the host responses of the wheat cultivars 'Loros x Koga' and 'Aus 10894'. Wheat cultivars 'Loros x Koga' and 'Aus 10894' were resistant to population GR. The population GR showed final population densities of 2.4 and 2.6 J2/ml soil, respectively.

The German population GR reproduced well on the wheat cultivars 'Capa', 'G7', 'G9', 'Giza 168', 'Iskamish', 'Psathias', 'Sakha 61', 'Sakha 8', 'Sakha 93' and 'Sakha 95' which are classified as susceptible. The local cultivars 'G9', 'Sahl 1' and 'Seds 1' were moderately susceptible to the GR population and showed *RS* of 46.0, 24.4 and 25.6%, respectively. The standard susceptible cultivar 'Capa' consistently showed the highest susceptibility to the German population with a *RS* of 100% followed by the Egyptian local cultivar 'Sakha 93' with a *RS* of 94.8%.

Pathotype determination of *H. avenae* populations

Pathotypes of six *H. avenae* populations were distinguished by testing for virulence against a number of wheat cultivars. **Table 4** is an updated matrix presenting the resistance reaction of cultivars from the International Test Assortment to different populations of *H. avenae* for defining cereal cyst nematode pathotypes **(Smiley *et al.,* 2011)**. The host responses of standard wheat cultivars to *H. avenae* populations under investigation were compared to the responses to several defined *H. avenae* pathotypes.

Following the combined pathotype scheme proposed by **Smiley *et al.,* (2011)**, the Egyptian populations and the German population belong to different pathotypes. The Egyptian populations showed behavior similar to that of pathotype Ha13, since the wheat cultivars 'Loros x Koga' and 'Aus 10894' were moderately resistant, and the cultivars 'Capa', 'Iskamish K-2 Light' and 'Psathias' were susceptible. On the other hand, the German population could be assigned to pathotype Ha11, since the cultivars 'Loros x Koga' and 'Aus 10894' were resistant while the cultivars 'Capa', 'Iskamish K-2 Light' and 'Psathias' were susceptible **(Table 4)**.

Table 4. Resistance reaction of wheat cultivars from the International Test Assortment to different populations of *H. avenae* for defining *H. avenae* pathotypes **(Smiley *et al.,* 2011)**.

Accession Name	**Code**	**Accession no.**	***H. avenae* pathotypes reaction***															
			Group 1								**Group 2**		**Group 3**	**Populations under investigation****					
			Ha 11	**Ha 21**	**Ha 31**	**Ha 41**	**Ha 51**	**Ha 61**	**Ha 71**	**Ha 81**	**Ha 12**	**Ha 22**	**Ha 13**	**AK**	**AS**	**EK**	**ES**	**SB**	**GR**
Aus 10894	AUS	NGB11099	R	-	-	R	-	R	R	R	R	S	(R)	**(R)**	**(R)**	**(R)**	**(R)**	**(R)**	**R**
Capa	CAP	NGB4823	S	S	-	S	-	S	S	S	S	S	S	**S**	**S**	**S**	**S**	**S**	**S**
Iskamish K-2-Light	ISK	NGB11091	S	-	-	R	-	(R)	(S)	R	(R)	S	S	**S**	**S**	**S**	**S**	**S**	**S**
Loros X Koga (63/1.7.15.12)	LOR	NGB11090	R	R	-	R	-	(R)	R	R	R	R	(R)	**(R)**	**(R)**	**(R)**	**(R)**	**(R)**	**R**
Psathias	PSA	NGB11098	S	-	-	S	-	-	R	R	S	R	S	**S**	**S**	**S**	**S**	**S**	**S**

* Phenotypic reaction: R = resistant; (R) = moderately resistant; (S) = moderately susceptible; and S = susceptible.

** AK = Abu Khalifah; AS = Abu Suwayr; EK = El Kasasen; ES = El Shark; SB = Serabeum and GR = Grafenreuth.

Damage caused by *H. avenae* populations on wheat cultivars under greenhouse conditions

- Reduction in grain yield

Grain yield of seven out of fourteen wheat cultivars tested was significantly reduced by *H. avenae* populations compared with the non-infested control **(Table 5)**. All nematode populations reduced the grain yield of 'Capa', 'G7', 'G9', 'Giza 168', 'Sakha 61', 'Sakha 8' and 'Sakha 95' by 20 to 42%. The highest reduction in the grain yield was recorded by the *H. avenae* population EK on the susceptible cultivar 'Capa'. Grain yield was not reduced in the resistant lines 'Loros x Koga' and 'Aus 10894' by the German nematode population (GR). Here, grain yield was only reduced by 0.1 and 0.5%, respectively.

- Tolerance of wheat cultivars to *H. avenae* populations

The tested wheat cultivars showed different degrees of tolerance to *H. avenae* populations **(Figure 1)**. Assessing the tolerance index according to **Dixon *et al.,* (1990)**, the local wheat cultivar 'Sakha 93' was the most tolerant wheat cultivar to all *H. avenae* populations with a tolerance index ranging between 0.2-0.3.

The wheat lines 'Loros x Koga' and 'Aus 10894' showed tolerance to the German population with a tolerance index 0 and 0.2, respectively. However, these wheat lines were less tolerant to the Egyptian populations as the tolerance index ranged between 0.2-0.8 and 0.4-0.8, respectively. The lines 'Iskamish K-2-Light' and 'Psathias' showed tolerance to the nematode populations and their tolerance index ranged between 0.3-0.5 and 0.3-0.6, respectively.

The tolerance index for the wheat cultivars 'Capa', 'Sakha 61' and 'Sakha 95' was between 0.5-0.7 and these cultivars showed less tolerance to the nematode populations followed by 'G7', 'G9', 'Giza 168' and 'Sakha 8'. The lowest tolerance (sensitive) was recorded by the wheat cultivars 'Seds 1' and 'Sahl 1' as their tolerance index ranged between 0.9-2 and 1-1.7, respectively.

Table 5. Effect of *H. avenae* populations on grain yield of different wheat cultivars.

Pop. / Cult.	Control	Abu Khalifah		Abu Suwayr		El Kasasen		El Shark		Serabeum		Grafenreuth	
	Yield (g)[a]	Yield (g)	*Red* (%) [b]	Yield (g)	*Red* (%)	Yield (g)	*Red* (%)	Yield (g)	*Red* (%)	Yield (g)	*Red* (%)	Yield (g)	*Red* (%)
Aus 10894	2.2 ± 0.5 **a**	2.1 ± 0.5 **a**	**4.8**	2.1 ± 0.6 **a**	**5.8**	2.0 ± 0.4 **a**	**8.1**	2.0 ± 0.4 **a**	**8.0**	2.1 ± 0.3 **a**	**7.0**	2.2 ± 0.6 **a**	**0.5**
Capa	2.4 ± 0.2 **a**	1.5 ± 0.5 **ab**	**38.3**	1.7 ± 0.5 **ab**	**30.7**	1.4 ± 0.3 **b**	**41.9**	1.6 ± 0.2 **ab**	**32.1**	1.6 ± 1.0 **ab**	**34.3**	1.5 ± 0.7 **ab**	**37.9**
Gemmeza 7	2.8 ± 0.5 **a**	2.2 ± 0.4 **ab**	**20.0**	2.0 ± 0.4 **b**	**27.5**	1.9 ± 0.2 **b**	**31.4**	1.9 ± 0.4 **b**	**30.4**	2.1 ± 0.4 **b**	**24.6**	2.3 ± 0.7 **ab**	**19.5**
Gemmeza 9	2.8 ± 0.6 **a**	2.0 ± 0.4 **b**	**28.7**	2.2 ± 0.7 **ab**	**22.6**	2.1 ± 0.4 **ab**	**25.0**	1.9 ± 0.8 **b**	**31.1**	2.2 ± 0.7 **ab**	**20.7**	2.2 ± 0.4 **ab**	**23.7**
Giza 168	2.1 ± 0.6 **a**	1.5 ± 0.4 **ab**	**29.4**	1.4 ± 0.5 **ab**	**32.8**	1.5 ± 0.8 **ab**	**27.0**	1.4 ± 0.4 **ab**	**30.8**	1.5 ± 0.4 **ab**	**25.8**	1.3 ± 0.2 **b**	**36.3**
Iskamish K-2	2.3 ± 0.5 **a**	2.1 ± 0.3 **a**	**8.1**	2.0 ± 0.4 **a**	**9.9**	1.9 ± 0.3 **a**	**16.4**	1.8 ± 0.4 **a**	**18.7**	1.9 ± 0.3 **a**	**13.8**	2.0 ± 0.3 **a**	**12.0**
Loros X Koga	2.1 ± 0.7 **a**	2.0 ± 0.2 **a**	**4.5**	2.1 ± 0.4 **a**	**2.5**	2.0 ± 0.3 **a**	**4.3**	2.0 ± 0.4 **a**	**5.6**	2.0 ± 0.4 **a**	**4.6**	2.1 ± 0.2 **a**	**0.1**
Psathias	2.2 ± 0.5 **a**	1.9 ± 0.2 **a**	**13.0**	1.9 ± 0.3 **a**	**10.4**	1.9 ± 0.5 **a**	**14.2**	1.8 ± 0.6 **a**	**18.0**	1.7 ± 0.4 **a**	**19.5**	1.9 ± 0.6 **a**	**12.6**
Sahl 1	2.4 ± 1.0 **a**	2.0 ± 0.3 **a**	**14.7**	2.0 ± 0.6 **a**	**16.5**	1.9 ± 0.5 **a**	**17.8**	1.8 ± 0.4 **a**	**24.1**	2.0 ± 0.2 **a**	**17.1**	1.8 ± 0.6 **a**	**23.5**
Sakha 61	2.2 ± 0.3 **a**	1.6 ± 0.1 **ab**	**25.8**	1.5 ± 0.3 **b**	**30.7**	1.6 ± 0.5 **b**	**28.9**	1.4 ± 0.3 **b**	**36.3**	1.5 ± 0.2 **b**	**32.4**	1.6 ± 0.2 **ab**	**26.4**
Sakha 8	2.1 ± 0.7 **a**	1.3 ± 0.3 **b**	**38.3**	1.4 ± 0.5 **b**	**33.9**	1.3 ± 0.5 **b**	**35.3**	1.2 ± 0.4 **b**	**42.2**	1.4 ± 0.2 **b**	**30.7**	1.2 ± 0.3 **b**	**41.8**
Sakha 93	2.8 ± 0.5 **a**	2.2 ± 0.2 **a**	**20.6**	2.3 ± 0.4 **a**	**19.4**	2.4 ± 0.4 **a**	**15.4**	2.5 ± 0.5 **a**	**12.9**	2.4 ± 0.3 **a**	**13.6**	2.4 ± 0.1 **a**	**15.9**
Sakha 95	2.8 ± 0.7 **a**	2.1 ± 0.1 **ab**	**24.5**	2.0 ± 0.1 **ab**	**27.8**	1.8 ± 0.3 **b**	**36.9**	2.0 ± 0.5 **b**	**30.2**	1.8 ± 0.3 **b**	**36.3**	2.0 ± 4.0 **ab**	**28.2**
Seds 1	2.3 ± 0.3 **a**	1.9 ± 0.2 **a**	**14.9**	1.9 ± 0.4 **a**	**15.1**	1.7 ± 0.4 **a**	**25.8**	1.8 ± 0.3 **a**	**20.5**	1.7 ± 0.5 **a**	**24.8**	1.8 ± 0.3 **a**	**19.2**

[a] Yield (g)= Means of grain yield/pot ± standard deviation. Means in a row followed by the same letter are not significantly different based on Tukey test ($P \leq 0.05$).

[b] *Red* (%) = Percentage of reduction in grain yield compared to control (0).

Red (%)=((*CP-IP*)/*CP*)*100, where *Red* (%)= percentage of reduction, *CP*= grain yield of control plant, *IP*= grain yield of infested plant.

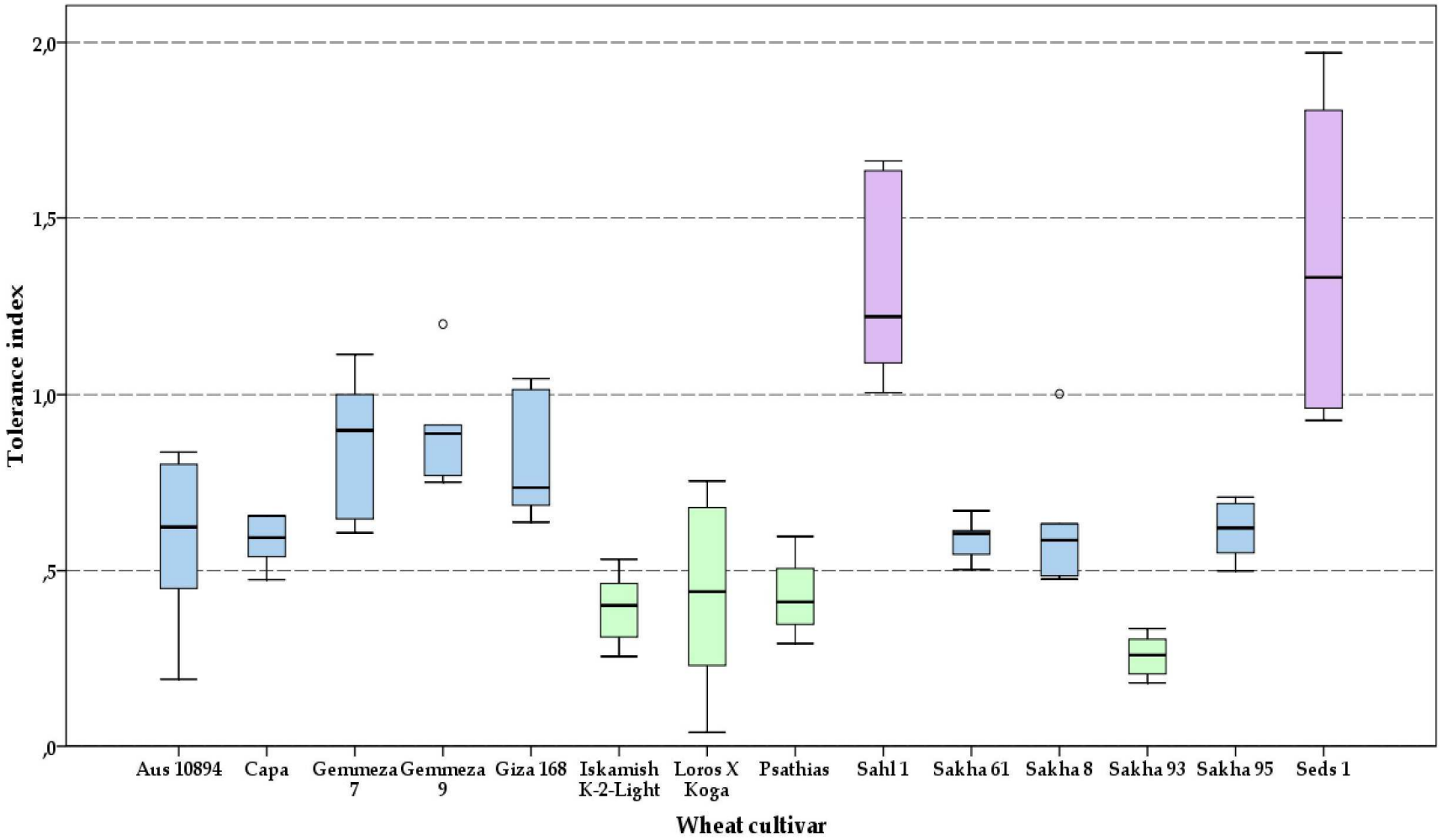

Figure 1. Box plot of the tolerance index of wheat cultivars to different populations of *H. avenae*. Tolerance index according to **Dixon *et al.*, (1990)** = ((GCP-GIP)/GCP)*100/Pf, where, GCP= grain yield of control plant, GIP= grain yield of infested plant, Pf= final population density of eggs and J2/ml soil. Tolerance ranking: tolerant (0-0.5); less tolerant (0.5-1) and sensitive (>1).

- Reduction in spike weight

The influence of *H. avenae* populations on the spike weight of different wheat cultivars is represented in **Table 6**. All populations of *H. avenae* significantly reduced spike weight of the wheat cultivars 'Capa', 'G7', 'G9', 'Giza 168', 'Iskamish K-2 light', 'Psathias', 'Sahl 1', 'Sakha 61', 'Sakha 8', 'Sakha 93', 'Sakha 95' and 'Seds 1' by 15.8 – 46%. The line 'Loros x Koga' was the only wheat cultivar in which the spike weight was not significantly reduced by all *H. avenae* populations. Here, percentages of reduction were less than 10 %.

- Reduction in shoot dry weight

Shoot dry weight of wheat cultivars 'Aus 10894' and 'Loros x Koga' was not reduced by the German nematode population of *H. avenae* **(Table 7)**. All populations of *H. avenae* significantly reduced shoot dry weight of wheat cultivars 'Capa', 'G7', 'G9', 'Giza 168', 'Iskamish K-2 light', 'Psathias', 'Sahl 1', 'Sakha 61', 'Sakha 8', 'Sakha 93', 'Sakha 95' and 'Seds 1' with percentages ranging between 16 – 49%.

- Reduction in root dry weight

The root dry weight of seven out of fourteen wheat cultivars was significantly reduced by *H. avenae* populations compared to the non-infested control **(Table 8)**. All nematode populations reduced the root dry weight of 'Capa', 'G7', 'G9', 'Giza 168', 'Sakha 61', 'Sakha 8' and 'Sakha 95' by 10 to 30%. Root weight of 'Aus 10894' and 'Loros x Koga' was not reduced by the German nematode population of *H. avenae* (reduction of 0.4 and 0.7%, respectively).

Table 6. Effect of *H. avenae* populations on spike weight of different wheat cultivars.

Pop. / Cult.	Control	Abu Khalifah		Abu Suwayr		El Kasasen		El Shark		Serabeum		Grafenreuth	
	Spike (g)[a]	Spike (g)	*Red* (%)[b]	Spike (g)	*Red* (%)	Spike (g)	*Red* (%)	Spike (g)	*Red* (%)	Spike (g)	*Red* (%)	Spike (g)	*Red* (%)
Aus 10894	3.5 ± 0.2 **a**	3.0 ± 0.3 **b**	**12.3**	3.0 ± 0.3 **b**	**12.6**	3.0 ± 0.2 **b**	**14.0**	3.0 ± 0.2 **b**	**13.9**	3.0 ± 0.2 **b**	**13.4**	3.5 ± 0.2 **a**	**0.5**
Capa	3.5 ± 0.1 **a**	2.4 ± 0.3 **b**	**33.5**	2.0 ± 0.3 **b**	**43.0**	1.9 ± 0.2 **b**	**45.9**	2.1 ± 0.2 **b**	**41.4**	2.1 ± 0.6 **b**	**40.0**	2.2 ± 0.1 **b**	**37.2**
Gemmeza 7	3.5 ± 0.2 **a**	2.5 ± 0.2 **b**	**27.8**	2.2 ± 0.3 **b**	**36.3**	2.3 ± 0.2 **b**	**35.2**	2.5 ± 0.2 **b**	**29.2**	2.5 ± 0.2 **b**	**29.4**	2.6 ± 0.2 **b**	**26.5**
Gemmeza 9	3.5 ± 0.3 **a**	2.3 ± 0.2 **b**	**35.4**	2.6 ± 0.1 **b**	**25.3**	2.5 ± 0.2 **b**	**28.8**	2.4 ± 0.2 **b**	**32.1**	2.5 ± 0.4 **b**	**29.5**	2.5 ± 0.3 **b**	**27.8**
Giza 168	3.4 ± 0.3 **a**	2.3 ± 0.2 **b**	**33.5**	2.0 ± 0.2 **b**	**40.6**	2.3 ± 0.1 **b**	**34.0**	2.1 ± 0.2 **b**	**37.6**	2.3 ± 0.3 **b**	**31.8**	2.1 ± 0.3 **b**	**38.2**
Iskamish K-2	3.2 ± 0.2 **a**	2.7 ± 0.1 **b**	**16.6**	2.7 ± 0.2 **b**	**17.1**	2.4 ± 0.2 **b**	**24.9**	2.5 ± 0.1 **b**	**24.1**	2.6 ± 0.2 **b**	**18.9**	2.6 ± 0.2 **b**	**19.2**
Loros X Koga	3.2 ± 0.3 **a**	2.9 ± 0.3 **a**	**7.1**	3.0 ± 0.2 **a**	**6.7**	2.9 ± 0.2 **a**	**8.9**	3.0 ± 0.2 **a**	**6.6**	2.9 ± 0.2 **a**	**9.0**	3.1 ± 0.3 **a**	**0.8**
Psathias	3.2 ± 0.2 **a**	2.7 ± 0.3 **ab**	**15.8**	2.7 ± 0.2 **b**	**17.0**	2.6 ± 0.2 **b**	**18.4**	2.6 ± 0.3 **b**	**19.9**	2.4 ± 0.2 **b**	**24.6**	2.5 ± 0.2 **b**	**23.0**
Sahl 1	3.5 ± 0.7 **a**	2.7 ± 0.2 **b**	**24.4**	2.5 ± 0.3 **b**	**30.1**	2.7 ± 0.2 **b**	**23.5**	2.6 ± 0.2 **b**	**27.9**	2.8 ± 0.1 **b**	**21.6**	2.6 ± 0.7 **b**	**25.8**
Sakha 61	3.2 ± 0.2 **a**	2.0 ± 0.2 **b**	**37.9**	1.9 ± 0.1 **b**	**40.8**	2.2 ± 0.2 **b**	**32.5**	2.2 ± 0.1 **b**	**31.7**	1.9 ± 0.1 **b**	**39.8**	2.1 ± 0.2 **b**	**34.2**
Sakha 8	3.2 ± 0.4 **a**	2.0 ± 0.2 **b**	**36.4**	2.0 ± 0.3 **b**	**35.1**	1.8 ± 0.1 **b**	**44.1**	1.7 ± 0.1 **b**	**45.6**	1.9 ± 0.1 **b**	**39.6**	1.9 ± 0.4 **b**	**39.3**
Sakha 93	3.6 ± 0.3 **a**	2.6 ± 0.2 **b**	**27.8**	2.6 ± 0.2 **b**	**26.2**	2.7 ± 0.3 **b**	**24.1**	2.8 ± 0.1 **b**	**23.2**	2.9 ± 0.1 **b**	**20.0**	2.9 ± 0.3 **b**	**19.5**
Sakha 95	3.3 ± 0.3 **a**	2.2 ± 0.2 **b**	**32.5**	2.1 ± 0.1 **b**	**36.6**	1.9 ± 0.2 **b**	**40.6**	2.1 ± 0.3 **b**	**34.7**	2.0 ± 0.1 **b**	**39.2**	2.3 ± 0.3 **b**	**29.5**
Seds 1	3.3 ± 0.1 **a**	2.6 ± 0.2 **b**	**20.5**	2.6 ± 0.2 **b**	**20.3**	2.5 ± 0.2 **b**	**24.4**	2.4 ± 0.2 **b**	**25.8**	2.3 ± 0.3 **b**	**28.9**	2.3 ± 0.1 **b**	**30.0**

[a] Spike (g) = Means of spike weight/pot ± standard deviation. Means in a row followed by the same letter are not significantly different based on Tukey test ($P \leq 0.05$).

[b] *Red* (%) = Percentage of reduction in the spike weight compared to control (0).

Red (%) = ((*CP*-*IP*)/*CP*)*100, where *Red* (%) = percentage of reduction, *CP*= spike weight of control plant, *IP*= spike weight of infested plant.

Table 7. Effect of *H. avenae* populations on shoot dry weight of different wheat cultivars.

Pop. / Cult.	Control	Abu Khalifah		Abu Suwayr		El Kasasen		El Shark		Serabeum		Grafenreuth	
	Shoot (g) [a]	Shoot (g)	*Red* (%) [b]	Shoot (g)	*Red* (%)	Shoot (g)	*Red* (%)	Shoot (g)	*Red* (%)	Shoot (g)	*Red* (%)	Shoot (g)	*Red* (%)
Aus 10894	9.8 ± 0.7 **a**	8.0 ± 0.7 **b**	**18.2**	8.2 ± 0.4 **b**	**16.6**	8.3 ± 0.4 **b**	**15.5**	7.4 ± 0.4 **b**	**24.5**	7.6 ± 0.4 **b**	**22.3**	9.8 ± 0.3 **a**	**0.4**
Capa	10.1 ± 0.6 **a**	6.1 ± 0.4 **b**	**39.6**	5.6 ± 0.4 **b**	**44.8**	5.8 ± 0.5 **b**	**42.0**	5.3 ± 0.1 **b**	**47.1**	5.9 ± 0.5 **b**	**41.1**	5.5 ± 0.5 **b**	**45.7**
Gemmeza 7	10.6 ± 0.5 **a**	6.4 ± 0.4 **d**	**39.1**	6.7 ± 0.4 **cd**	**37.0**	6.9 ± 0.2 **bcd**	**34.9**	7.4 ± 0.5 **bc**	**30.2**	7.0 ± 0.3 **bcd**	**33.8**	7.5 ± 0.5 **b**	**29.1**
Gemmeza 9	10.6 ± 0.6 **a**	6.7 ± 0.4 **b**	**37.0**	7.1 ± 0.7 **b**	**32.4**	6.3 ± 0.4 **b**	**39.9**	6.9 ± 0.8 **b**	**35.0**	6.5 ± 0.8 **b**	**38.3**	7.0 ± 0.3 **b**	**33.6**
Giza 168	10.3 ± 0.6 **a**	6.9 ± 0.3 **b**	**33.2**	5.9 ± 0.2 **cd**	**42.6**	6.6 ± 0.7 **bcd**	**35.8**	6.2 ± 0.4 **bcd**	**39.7**	5.8 ± 0.4 **d**	**44.0**	6.7 ± 0.2 **bc**	**34.8**
Iskamish K-2	10.0 ± 0.5 **a**	7.9 ± 0.3 **b**	**21.1**	7.2 ± 0.5 **bc**	**27.6**	7.3 ± 0.4 **bc**	**26.6**	7.0 ± 0.5 **c**	**29.6**	8.1 ± 0.4 **b**	**19.4**	7.7 ± 0.3 **bc**	**23.0**
Loros X Koga	10.0 ± 0.3 **a**	8.4 ± 0.4 **b**	**16.5**	8.2 ± 0.4 **b**	**18.6**	8.1 ± 0.4 **b**	**19.3**	7.6 ± 0.3 **b**	**24.0**	7.7 ± 0.6 **b**	**23.2**	10 ± 0.6 **a**	**0.7**
Psathias	9.9 ± 0.5 **a**	7.8 ± 0.5 **b**	**21.1**	7.4 ± 0.5 **b**	**25.2**	7.5 ± 0.5 **b**	**24.3**	7.1 ± 0.6 **b**	**28.1**	7.3 ± 0.4 **b**	**26.1**	7.6 ± 0.6 **b**	**23.0**
Sahl 1	10.4 ± 1.2 **a**	8.0 ± 0.2 **b**	**23.6**	7.3 ± 0.4 **b**	**30.6**	7.6 ± 0.5 **b**	**27.4**	6.9 ± 0.4 **b**	**33.5**	7.9 ± 0.2 **b**	**24.7**	7.5 ± 0.6 **b**	**28.5**
Sakha 61	9.9 ± 0.4 **a**	6.1 ± 0.4 **b**	**38.4**	6.5 ± 0.3 **b**	**34.9**	5.4 ± 0.2 **c**	**45.2**	6.2 ± 0.1 **b**	**37.6**	5.5 ± 0.2 **c**	**44.5**	6.3 ± 0.1 **b**	**36.4**
Sakha 8	9.8 ± 0.8 **a**	5.4 ± 0.3 **b**	**45.1**	5.9 ± 0.5 **b**	**40.0**	5.0 ± 0.1 **b**	**48.7**	5.6 ± 0.2 **b**	**42.9**	5.1 ± 0.5 **b**	**47.4**	5.7 ± 0.2 **b**	**41.9**
Sakha 93	10.7 ± 0.2 **a**	7.7 ± 0.6 **b**	**28.0**	8.7 ± 0.6 **b**	**18.7**	8.2 ± 1.5 **b**	**23.0**	7.4 ± 0.3 **b**	**30.4**	8.4 ± 0.5 **b**	**21.1**	8.6 ± 0.7 **b**	**19.6**
Sakha 95	10.0 ± 0.7 **a**	6.6 ± 0.5 **b**	**34.3**	6.1 ± 0.1 **bcd**	**39.1**	5.3 ± 0.2 **d**	**46.9**	5.8 ± 0.4 **cd**	**42.4**	6.4 ± 0.1 **bc**	**36.4**	5.9 ± 0.4 **bcd**	**41.2**
Seds 1	10.5 ± 0.5 **a**	7.9 ± 0.5 **b**	**24.4**	8.0 ± 0.6 **b**	**24.2**	6.6 ± 0.3 **c**	**37.0**	7.0 ± 0.4 **bc**	**32.9**	7.4 ± 0.3 **bc**	**29.1**	7.2 ± 0.6 **bc**	**31.8**

[a] Shoot (g) = Means of shoot dry weight ± standard deviation. Means in a row followed by the same letter are not significantly different based on Tukey test ($P \leq 0.05$).

[b] *Red* (%) = Percentage of reduction in the soot dry weight compared to control (0).

Red (%) = ((*CP-IP)/CP*)*100, where *Red* (%) = percentage of reduction, *CP*= shoot dry weight of control plant, *IP*= shoot dry weight of infested plant.

Table 8. Effect of *H. avenae* populations on root dry weight of different wheat cultivars.

Pop. / Cult.	Control	Abu Khalifah		Abu Suwayr		El Kasasen		El Shark		Serabeum		Grafenreuth	
	Root (g) [a]	Root (g)	*Red* (%) [b]	Root (g)	*Red* (%)	Root (g)	*Red* (%)	Root (g)	*Red* (%)	Root (g)	*Red* (%)	Root (g)	*Red* (%)
Aus 10894	3.1 ± 0.1 **a**	3.0 ± 0.2 **a**	**1.2**	2.9 ± 0.2 **a**	**5.7**	3.0 ± 0.2 **a**	**3.1**	3.0 ± 0.2 **a**	**3.8**	3.0 ± 0.3 **a**	**2.6**	3.1 ± 0.1 **a**	**0.7**
Capa	3.2 ± 0.1 **a**	2.4 ± 0.2 **b**	**24.7**	2.6 ± 0.2 **b**	**18.3**	2.2 ± 0.4 **b**	**29.7**	2.5 ± 0.3 **b**	**20.6**	2.4 ± 0.2 **b**	**25.2**	2.5 ± 0.1 **b**	**19.5**
Gemmeza 7	3.0 ± 0.3 **a**	2.4 ± 0.2 **b**	**20.0**	2.5 ± 0.1 **ab**	**15.8**	2.4 ± 0.3 **b**	**21.5**	2.6 ± 0.2 **ab**	**15.1**	2.7 ± 0.2 **ab**	**10.7**	2.6 ± 0.3 **ab**	**14.8**
Gemmeza 9	3.0 ± 0.2 **a**	2.7 ± 0.3 **ab**	**9.6**	2.6 ± 0.3 **ab**	**12.6**	2.3 ± 0.3 **b**	**21.7**	2.5 ± 0.3 **ab**	**15.9**	2.4 ± 0.2 **b**	**20.6**	2.6 ± 0.2 **ab**	**14.8**
Giza 168	3.1 ± 0.1 **a**	2.4 ± 0.1 **b**	**21.9**	2.6 ± 0.2 **ab**	**15.3**	2.3 ± 0.5 **b**	**24.8**	2.6 ± 0.2 **ab**	**16.0**	2.4 ± 0.3 **b**	**22.7**	2.5 ± 0.1 **b**	**19.6**
Iskamish K-2	3.0 ± 0.3 **a**	2.8 ± 0.1 **a**	**4.7**	2.7 ± 0.2 **a**	**9.4**	2.7 ± 0.2 **a**	**7.7**	2.6 ± 0.2 **a**	**11.7**	2.7 ± 0.2 **a**	**7.1**	2.7 ± 0.3 **a**	**10.0**
Loros X Koga	3.1 ± 0.2 **a**	3.0 ± 0.1 **a**	**3.2**	3.0 ± 0.1 **a**	**2.5**	2.9 ± 0.2 **a**	**5.2**	2.9 ± 0.2 **a**	**5.4**	3.0 ± 0.1 **a**	**4.8**	3.1 ± 0.2 **a**	**0.3**
Psathias	3.1 ± 0.2 **a**	2.8 ± 0.2 **a**	**8.3**	2.8 ± 0.2 **a**	**10.6**	2.9 ± 0.2 **a**	**5.7**	2.8 ± 0.4 **a**	**8.1**	2.9 ± 0.3 **a**	**5.1**	2.9 ± 0.2 **a**	**4.7**
Sahl 1	3.0 ± 0.3 **a**	2.8 ± 0.3 **a**	**7.6**	2.7 ± 0.3 **a**	**11.1**	2.6 ± 0.2 **a**	**13.8**	2.6 ± 0.2 **a**	**13.0**	2.7 ± 0.2 **a**	**9.3**	2.5 ± 0.3 **a**	**15.9**
Sakha 61	3.2 ± 0.1 **a**	2.5 ± 0.2 **b**	**19.1**	2.3 ± 0.2 **b**	**26.0**	2.4 ± 0.3 **b**	**22.9**	2.6 ± 0.2 **b**	**16.3**	2.5 ± 0.2 **b**	**20.7**	2.5 ± 0.1 **b**	**19.7**
Sakha 8	3.1 ± 0.1 **a**	2.4 ± 0.1 **b**	**23.2**	2.4 ± 0.2 **b**	**22.5**	2.2 ± 0.4 **b**	**28.5**	2.2 ± 0.2 **b**	**27.0**	2.3 ± 0.2 **b**	**24.9**	2.3 ± 0.1 **b**	**25.0**
Sakha 93	3.2 ± 0.1 **a**	3.0 ± 0.2 **a**	**4.7**	3.0 ± 0.2 **a**	**5.4**	2.9 ± 0.1 **a**	**9.5**	2.9 ± 0.2 **a**	**10.1**	3.0 ± 0.1 **a**	**6.1**	3.0 ± 0.1 **a**	**7.0**
Sakha 95	3.1 ± 0.2 **a**	2.5 ± 0.3 **b**	**18.1**	2.6 ± 0.1 **b**	**15.7**	2.3 ± 0.2 **b**	**26.0**	2.5 ± 0.3 **b**	**19.5**	2.3 ± 0.2 **b**	**25.8**	2.5 ± 0.2 **b**	**18.6**
Seds 1	3.1 ± 0.1 **a**	2.9 ± 0.1 **a**	**5.5**	2.8 ± 0.2 **a**	**8.6**	2.7 ± 0.4 **a**	**13.4**	2.5 ± 0.5 **a**	**17.7**	2.6 ± 0.2 **a**	**16.4**	2.8 ± 0.1 **a**	**9.8**

[a] Root (g) = Means of root dry weight ± standard deviation. Means in a row followed by the same letter are not significantly different based on Tukey test ($P \leq 0.05$).

[b] *Red* (%) = Percentage of reduction in the root dry weight compared to control (0).

Red (%) = ((*CP-IP)/CP*)*100, where *Red* (%) = percentage of reduction, *CP*= root dry weight of control plant, *IP*= root dry weight of infested plant.

DISCUSSION

The *H. avenae* populations from the five regions of Ismailia province have the same virulence phenotype. It may be that these populations are indigenous or may have been introduced from the same source **(Al-Hazmi *et al*., 2001)**. Nevertheless, the Egyptian populations and the German population showed different responses on the differentials which means that they belong to different pathotypes. This variation was probably associated with the different climates in which these populations developed in two different geographical regions **(Haddadi *et al.,* 2013)**. The Egyptian populations were defined as pathotype Ha13 similar to the Australian populations of *H. avenae* which previously defined by **Brown and Meagher, (1970); O'Brien and Fisher, (1979)**.

The German population could be assigned to pathotype Ha11. Cultivars such 'Loros x Koga' and 'Aus 10894' which contain the *Cre 1* gene, have consistently been effective against the European populations of *H. avenae* in several countries like Denmark **(Anderson, 1959)**, Sweden **(Wahlstedt, 1967)**, United Kingdom **(Fiddian and Kimber, 1964; Saynor, 1975)**, the Netherlands **(Kort *et al.,* 1964)**, Germany **(Lücke, 1976)** and France **(Rivoal, 1977)** where pathotype Ha11was detected.

Resistance and tolerance are genetically independent, and both are required for optimal performance of crop plants under pest pressure. Resistance will reduce population densities and tolerance will help to recover from the damaging effects of nematode attack and yield well **(Trudgill, 1991)**. Ideally the resistance should be combined with tolerance. The Egyptian local cultivar 'Sakha 93' was the only wheat cultivar that shows a tolerance to all *H. avenae* populations, as no reduction of grain yield was detected in spite of its high relative susceptibility to the *H. avenae* under greenhouse conditions. 'Sakha 93' is a widely grown wheat cultivar in Egypt with high yields and tolerance to water stress **(El-Ashry and El-Kholy, 2005)**. In a field experiment, 'Sakha 93' surpasses the other tested varieties ('Gemmeiza 7', 'Gemmeiza 9', 'Gemmeiza 10', 'Sakha 92', 'Sakha 94', 'Giza 164' and 'Giza 168') in the harvested yield **(Ibrahim *et al.,* 2011)**.

Continued cultivation of a tolerant but susceptible cultivar may ultimately build up nematode populations to very high levels, resulting in damage to even tolerant genotypes. In contrast, sowing an intolerant but resistant variety into field with high nematode population densities can result in partial crop failure **(Smiley *et al.,* 2013)**. It should be noted that there was no resistance in any of the tested wheat cultivars to the Egyptian populations. To initiate breeding for resistance and tolerance to cereal cyst nematode in Egypt, the most urgent need is to obtain parent material suitable for production of a resistant and tolerant cultivar of wheat.

There is a need to search for sources of resistance to this nematode among Egyptian wheat germplasm or to introduce resistant germplasm from another cereal for Egyptian breeding program. Resistance genes like *Cre3* from *Aegilops tauschii* and *Cre6* from *A. ventricosa* 5Nv provide resistance against pathotype Ha13 **(Ogbonnaya *et al.,* 2001)**. *Cre5* from *A. ventricosa* confers partial resistance to Australian (Ha13) pathotypes **(Rivoal *et al.,* 1993, Jahier *et al.,* 2001, Ogbonnaya *et al.,* 2001)**. Wheat cultivars carrying *Cre8* from *T. aestivum* exhibit partial resistance to Ha13. **Ogbonnaya *et al.,* (2001)** reported that the inhibition of Ha13 nematode reproduction was ranked in the order *Cre6* > *Cre3*> *Cre8* and *Cre5*.

The Egyptian populations of *H. avenae* were defined as pathotype Ha13 which is widespread in southeastern Australia. Due to the deployment of resistant cultivars, population densities of *H. avenae* in Australia have strongly declined **(Ogbonnaya *et al.,* 2009).** One effective source of resistance probable will be sharing such known resistant germplasm from Australia to determine the effectiveness of such resistance in Egypt. Another effective source of resistance will be the pathotype (Ha13) specific resistance genes (*Cre3* from *A. tauschii* and *Cre6* from *A. ventricosa* 5Nv) which could be deployed by pyramiding diverse sources of resistance genes. This may followed by the incorporation of the resistance into locally adapted cultivars which are then used in integrated *H. avenae* management programs.

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CHAPTER 5

Influence of population density of cereal cyst nematode populations (*Heterodera avenae* Wollenweber) on the nematode reproduction and damage to wheat cultivars

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ABSTRACT

The cereal cyst nematode (CCN) *Heterodera avenae* Wollenweber, is an important nematode pest of wheat. The extent of damage caused by cereal cyst nematodes depends among others on population densities in the soil. The objective of this work was to determine the influence of initial nematode population density of *H. avenae* from Egypt on the yield as well as on other plant growth parameters of different wheat cultivars and on nematode reproduction. The final nematode population density was positively correlated with the initial population density on all the tested wheat cultivars. A negative relationship between the initial population density and the rate of reproduction was observed. Plant growth (grain yield, spike weight, shoot dry weight and root dry weight) was negatively affected by increasing the initial population (P_i) density of *H. avenae*. The reduction in the grain yield of the Egyptian wheat cultivars by *H. avenae* ranged between 16-28% at a P_i -value of 5 J2/ml soil, 20-34% at a P_i –value of 10 J2/ml soil and 24-40% at a P_i -value of 20 J2/ml soil. The local wheat cultivar 'Sakha 93' showed tolerance in spite of his high relative susceptibility to the nematode at a P_i -value of 5 and 10 J2/ml soil as there was no significant reduction in grain yield. This study indicates that Egyptian populations of *H. avenae* are serious pests of Egyptian wheat cultivars and potentially a limiting factor in the production of wheat in Egypt.

Keywords - *Heterodera avenae*, wheat, initial density, final density, reproduction, grain yield, resistance, tolerance.

INTRODUCTION

The cereal cyst nematode (CCN) *Heterodera avenae* Wollenweber, causes significant economic losses in cereal crops. In Egypt, *H. avenae* has been reported in wheat fields for the first time by **Ibrahim *et al.,* 1986** on barley and wheat in the Nile Delta and other localities of Northern Egypt. In **2007, Ibrahim and Handoo** reported the occurrence of *H. avenae* on Egyptian wheat in Alexandria and El-Behera governorates. Recently, *H. avenae* was detected in wheat fields of Abu Khalifah, Abu Suwayr, El Kasasen, El Shark (West Sinai), and Serabeum regions in Ismailia province **(chapter 2)**.

Reductions of wheat yields by *H. avenae* have been reported from different regions of the world. In Morocco, *H. avenae* caused wheat grain yield losses of about 40-50% **(Rammah, 1994)**, up to 90% in Spain **(Romero *et al.,* 1988)** and as high as 96% in Tunisia **(Namouchi-Kachouri *et al.,* 2009)**. In Turkey, significant yield losses (average 42%) in several rain-fed winter wheat locations have been reported **(Nicol *et al.,* 2005)**. Reductions of wheat yield by *H. avenae* have been reported from Libya **(Siddiqui and Khan, 1986)**, France **(Rivoal and Sarr, 1988)** and Italy **(Greco *et al.,* 1993).** In Egypt, since the distribution of CCN in the other wheat growing regions is still unknown, and the local wheat cultivars which are susceptible to the nematode, are grown in monoculture by most local growers; the problem of *H. avenae* is becoming more serious.

In Australia, *H. avenae* decreased the yield of wheat by 20% at an initial population density (P_i) of 2 eggs and juveniles/g soil, and 40% at a P_i -value of 16 eggs and juveniles/g soil **(Meagher and Brown, 1974).** In Asia, the damage threshold of *H. avenae* in the temperate semi-arid regions of India is considered to be 5-20 eggs and juveniles/g soil (P_i) for wheat **(Gill and Swarup, 1971; Dhawan and Nagesh, 1987). Mathur *et al.,* (1986)** reported loss in wheat ranging from 32.4 to 66.5%in India, due to initial nematode populations densities (P_i) of *H. avenae* varying from 4.6 to 10.6 eggs/ml soil. In Egypt, nematode density in infested fields may reach 40 eggs and juveniles/ml soil **(Chapter 2).** Reductions in wheat yield under greenhouse conditions

at an initial population density of 5 eggs and juveniles/ml of soil may reach 42% **(Chapter 4)**.

The objective of this work was to determine the influence of different initial nematode population densities (0, 5, 10, 20 eggs+ juveniles/ml soil) of *H. avenae* on plant growth and yield of wheat cultivars and on nematode reproduction.

MATERIALS AND METHODS

Nematode populations

One Egyptian population of *H. avenae* from El Shark (West Sinai) in Ismailia province was used to examine the effect of increasing initial population densities of *H. avenae* on plant growth parameters of wheat cultivars and on nematode reproduction. This population was classified as pathotype Ha13 **(Chapter 4)**. Nematode cysts were dried at room temperature (20±2°C) and kept at 7°C until further use. Nematodes were reared on the Egyptian wheat (*Triticum aestivum*) cultivar 'Sakha 93' and newly formed cysts were used in the experiment. Cysts were squashed according to **Seinhorst and Den Ouden (1966)** and the total numbers of eggs and second stage juveniles (J2) per cyst were counted to determine cyst contents. Cyst content was on average 131.3±12.1 eggs and J2/cyst.

Plant materials

Three standard wheat cultivars ('Aus 10894', 'Capa' and 'Iskamish K-2-Light') from the International Test Assortment in addition to three locally-grown bread wheat cultivars ('Gemmeza 9', 'Sahl 1' and 'Sakha 93') from Egypt were used in this study.

Experimental set-up

Plastic pots (500ml) were filled with a sterilized soil mixture (2 loam: 1 field soil), fertilized with a granular fertilizer (Osmocote Exact Standard® 1.5g/kg soil). Cysts of *H. avenae* were added to the pots to give initial population densities (P_i) of 0, 5, 10, 20 eggs and juveniles per ml soil. Five pots were used as replicates for each initial nematode population density (P_i). Each pot contained five seedlings of the respective wheat cultivar. For each cultivar, five pots of non infested soil served as control. Plants were grown in a greenhouse at 15±3°C (16 h light/8 h dark) and watered as necessary with tap water.

Data collection and analysis

After 4 months, the final nematode numbers (P_f) were determined. Cysts were extracted from the soil using the floatation technique **(Shepherd, 1986)**. Counting and separation of cysts from soil debris and other organic materials retained on the filter paper were carried out at 25x magnification under a stereoscopic binocular (Leica MZ8). Cysts were squashed according to **Seinhorst and Den Ouden (1966)** and the number of eggs and juveniles were counted. The nematode reproduction factor (*Rf*) for each replicate was calculated as follows: $Rf = P_f/P_i$; where P_f = final population density of eggs and J2/ml soil; P_i = initial population density of eggs and J2/ml soil.

Resistance was assessed as relative susceptibility (*RS*) to the standard susceptible control cultivar 'Capa' (*RS*= P_f on the test cultivar/P_f on susceptible control 'Capa')*100, where *Pf*= final population density of eggs and J2/ml soil. A rating system based on the relative susceptibility was used to characterize the host response of different wheat cultivars **(Lücke, 1976)**. Cultivars with *RS* less than 5% were considered resistant. The cultivars were considered moderately resistant if *RS* was between 6-20%. Cultivars with 21-50% relative susceptibility were considered moderately susceptible while cultivars with *RS* > 51% were recorded as susceptible cultivars **(Lücke, 1976).**

Growth parameters (shoot dry weight, root dry weight, spike weight and grain yield per pot) were recorded to determine the damage potential of *H. avenae* populations on wheat cultivars. The percentages of reduction in different plant growth parameters were calculated as follows: *Red* (%) = ((*CP-IP*)/*CP*)*100, where *Red* (%) = percentage of reduction, *CP*= growth parameters of control plant, *IP*= growth parameters of infested plant. The tolerance index of a cultivar at each nematode initial population density was calculated as follows: *TI*= ((*GCP-GIP*)/*GCP*)*100/P_f, where *TI*= tolerance index, *GCP*= grain yield of control plant, *GIP*= grain yield of infested plant, P_f= final population density of eggs and J2/ml soil. Wheat cultivars with *TI* less than 0.5 considered tolerant. Wheat cultivars were less tolerant when *TI* was between 0.5 – 1. Wheat cultivars with *TI* higher than 1, were considered sensitive to *H. avenae* populations **(Dixon *et al.,* 1990)**.

Levene's test was used to test homogeneity of variances. Data were analyzed using ANOVA (SPSS version 19.0, IBM Corporation, New Orchard Road Armonk, New York, United States). Means were separated using Tukey HSD test at $P \leq 0.05$. Regression analyses were performed on the data to describe the relation between the nematode initial population density and nematode final population density; nematode reproduction factor; relative susceptibility of wheat cultivars and different plant growth parameters.

RESULTS

Effect of increasing initial population densities of *H. avenae* on the final population densities

Regression analyses showed that final population densities (P_f) were positively correlated with initial population densities (P_i) on the tested wheat cultivars **(Figure 1)**. The Egyptian *H. avenae* population (ES) reproduced on all tested wheat cultivars. As the P_i of *H. avenae* increased, the P_f *was* significantly higher on all the cultivars except 'Aus 10894' and 'Sakha 93' **(Table 1)**. The P_f of *H. avenae* on the cultivars 'Aus 10894' and 'Sakha 93' were not significantly different at all P_i levels and ranged between 13.5 – 21.5 and 63.4 – 70.6 J2/ml soil, respectively. At a P_i of 5 and 10 J2/ml soil, the P_f of *H. avenae* on the cultivar 'Iskamish K-2-Light' were not significantly different from each other, while the final population was significantly higher at a P_i of 20 J2/ml soil. The P_f on the cultivars 'Capa', 'Gemmeza 9' and 'Sahl 1' increased significantly with increasing P_i levels. The highest P_f was recorded at a P_i of 20 J2/ml soil on the cultivar 'Capa' with 117 J2/ml soil followed by the cultivars 'Gemmeza 9' and 'Sahl 1' with P_f of 83 and 74.3 J2/ml soil, respectively.

Effect of increasing initial population densities of *H. avenae* on the nematode reproduction factor

A negative relationship between initial population densities (P_i) and reproduction factor (*Rf*) of *H. avenae* was detected on all tested wheat cultivars **(Figure 2)**. The reproduction factor of *H. avenae* decreased significantly on the tested wheat cultivars in response to increasing nematode P_i except on the cultivar Sahl 1 **(Table 1)**. Nematode reproduction factor at a P_i of 5 and 10 J2/ml soil ranged between 2.7 – 14.1 and 1.7 – 9.1, respectively. The highest *Rf* at a P_i of 5 and 10 J2/ml soil was reported on the cultivar 'Capa' followed by the cultivar 'Sakha 93' while the lowest *Rf* was recorded on the cultivar 'Aus 10894' followed by the cultivar 'Sahl 1'. The reproduction factor of *H. avenae* on wheat cultivars ranged between 1.1- 5.8 at a P_i of 20 J2/ml soil. The highest *Rf* at a P_i of 20 J2/ml soil was recorded on the cultivar 'Capa' followed by the

cultivar 'Gemmeza 9' while lowest *Rf* was recorded on the cultivars 'Aus 10894' and 'Iskamish K-2-Light'.

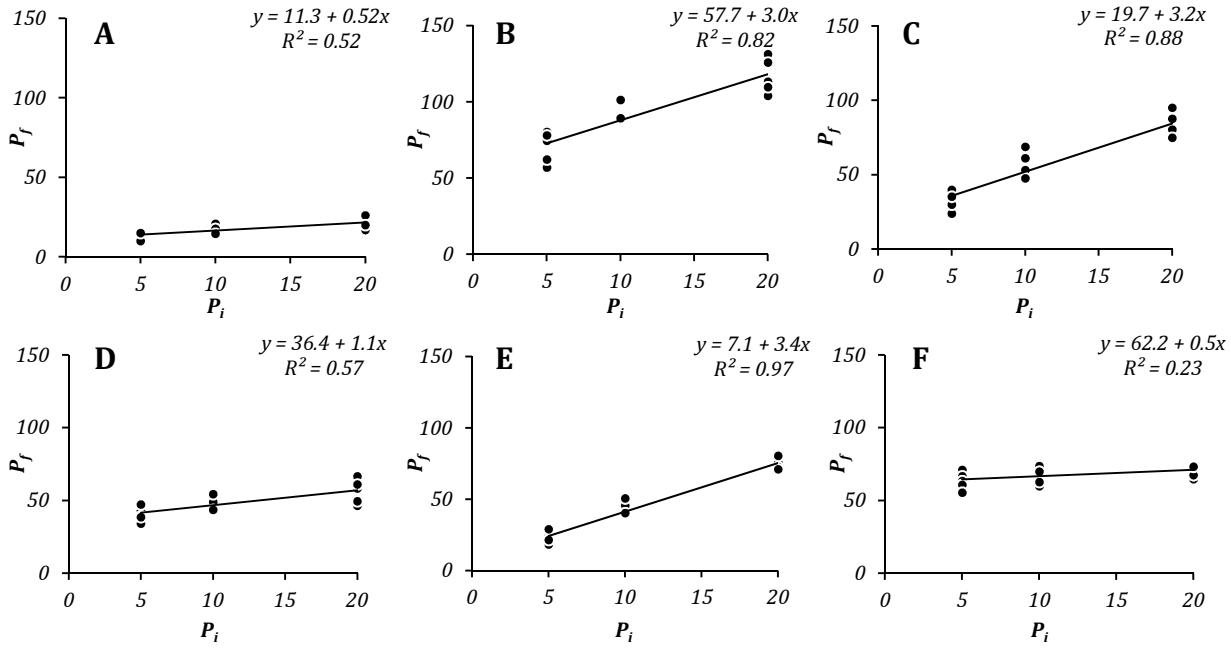

Figure 1. Relationship between initial population densities P_i (eggs+J2/ml soil) and final population densities P_f (eggs+J2/ml soil) of *Heterodera avenae* on wheat cultivars: 'Aus 10894' **(A)**; 'Capa' **(B)**; 'Gemmeza 9' **(C)**; 'Iskamish K-2-Light' **(D)**; 'Sahl 1' **(E)**; 'Sakha 93' **(F)**.

Table 1. Effect of different initial population densities (P_i) of *Heterodera avenae* on nematode final population density (P_f), nematode reproduction factor (*Rf*) and relative susceptibility (*RS*) of wheat cultivars.

Cultivars	P_i = **5** J2/ml soil				P_i = **10** J2/ml soil				P_i = **20** J2/ml soil			
	Pf [a]	***Rf*** [b]	***RS*** [c]	***Rank*** [d]	***Pf***	***Rf***	***RS***	***Rank***	***Pf***	***Rf***	***RS***	***Rank***
Aus 10894	13.5 ± 2.5 **a**	2.7 **a**	19.2	**(R)**	17.0 ± 2.5 **a**	1.7 **b**	18.5	**(R)**	21.5 ± 4.9 **a**	1.1 **c**	18.4	**(R)**
Capa	70.4 ± 10.3 **a**	14.1 **a**	100	**S**	91.5 ± 5.5 **b**	9.1 **b**	100	**S**	116.8 ± 11.4 **c**	5.8 **c**	100	**S**
Gemmeza 9	33.1 ± 6.3 **a**	6.6 **a**	47.0	**(S)**	56.2 ± 8.7 **b**	5.6 **ab**	61.4	**S**	83.0 ± 8.2 **c**	4.2 **b**	71.1	**S**
Iskamish K-2	40.4 ± 4.9 **a**	8.1 **a**	57.4	**S**	48.4 ± 4.2 **ab**	4.8 **b**	53.0	**S**	56.4 ± 8.3 **b**	2.8 **c**	48.3	**(S)**
Sahl 1	22.4 ± 4.3 **a**	4.5 **a**	31.8	**(S)**	43.7 ± 4.5 **b**	4.4 **a**	47.8	**(S)**	74.3 ± 4.0 **c**	3.7 **a**	63.6	**S**
Sakha 93	63.4 ± 5.9 **a**	12.7 **a**	90.1	**S**	68.1 ± 6.6 **a**	6.8 **b**	74.4	**S**	70.6 ± 4.3 **a**	3.5 **c**	60.5	**S**

[a] P_f = final population density of eggs and J2/ml soil.

[b] *Rf* (Reproduction factor) = P_f (Final population density)/ P_i (Initial population density).

P_f and *Rf* means in a row followed by the same letter are not significantly different based on Tukey test (P ≤ 0.05).

[c] *RS* (Relative susceptibility %) = P_f on the test cultivar/P_f on susceptible control 'Capa')*100.

[d] *Rank* (Resistance ranking) according to Lücke (1976): R, resistant (0-5%); (R), moderately resistant (6-20%); (S), moderately susceptible (21-50%); and S, susceptible (>51%).

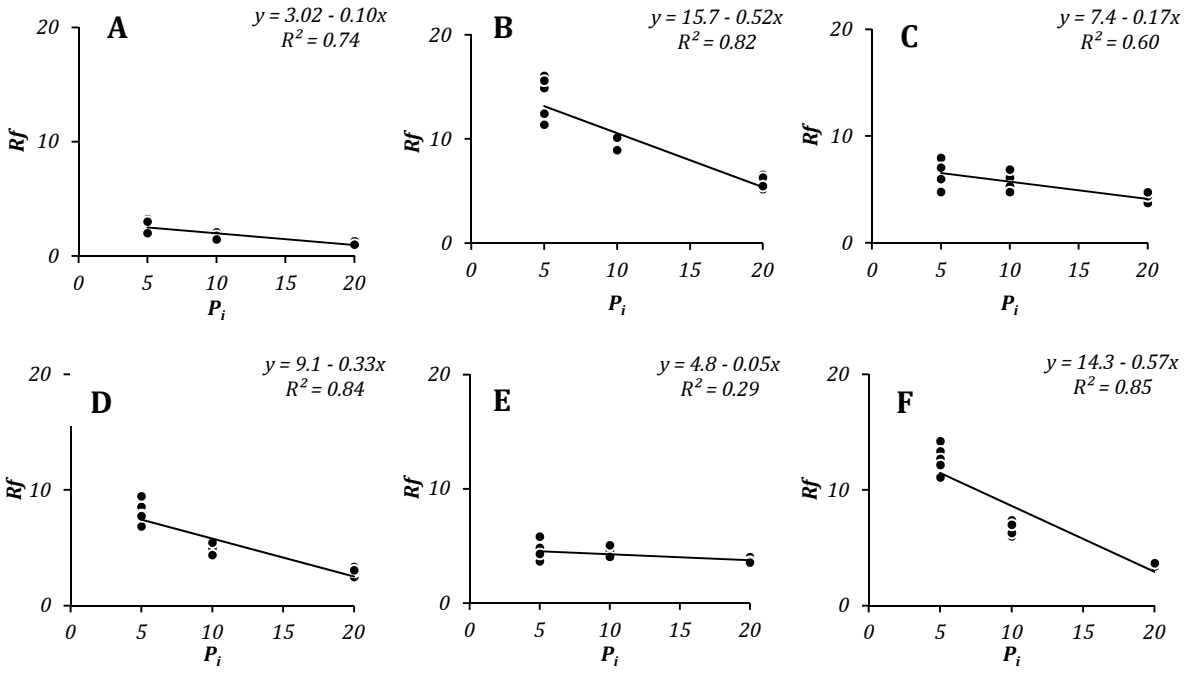

Figure 2. Relationship between initial population density P_i (eggs+J2/ml soil) and reproduction factor *Rf* of *Heterodera avenae* on wheat cultivars: Aus 10894 **(A)**; Capa **(B)**; Gemmeza 9 **(C)**; Iskamish K-2-Light **(D)**; Sahl 1 **(E)**; Sakha 93 **(F)**.

Effect of increasing initial population densities of *H. avenae* on the relative susceptibility of wheat cultivars

The relative susceptibilities (*RS*) of the tested wheat cultivars changed in response to increasing initial population densities (P_i) (**Figure 3)**. A positive correlation between the P_i and *RS* was detected on the wheat cultivars 'Gemmeza 9' and 'Sahl 1', while a negative relationship between the P_i and *RS* was detected on the wheat cultivars 'Aus 10894', 'Iskamish K-2-Light' and 'Sakha 93'. At a P_i of 5 J2/ml soil, 'Gemmeza 9' and 'Sahl 1' were moderately susceptible cultivars to *H. avenae* with a *RS* of 47 and 31.8 %, respectively (**Table 1**). While at a P_i of 10, Gemmeza 9 was classified as susceptible. The cultivar 'Sahl 1' changed from moderately susceptible to susceptible. The cultivar 'Aus 10894' was moderately resistant at all P_i levels of *H. avenae.* The cultivar 'Sakha 93' was susceptible at all P_i levels. Different response from the wheat cultivar 'Iskamish K-2-Light' was recorded at all P_i levels. This cultivar was

susceptible at a P_i of 5 and 10 J2/ml soil, while it was classified as moderately susceptible at P_i of 20 J2/ml soil.

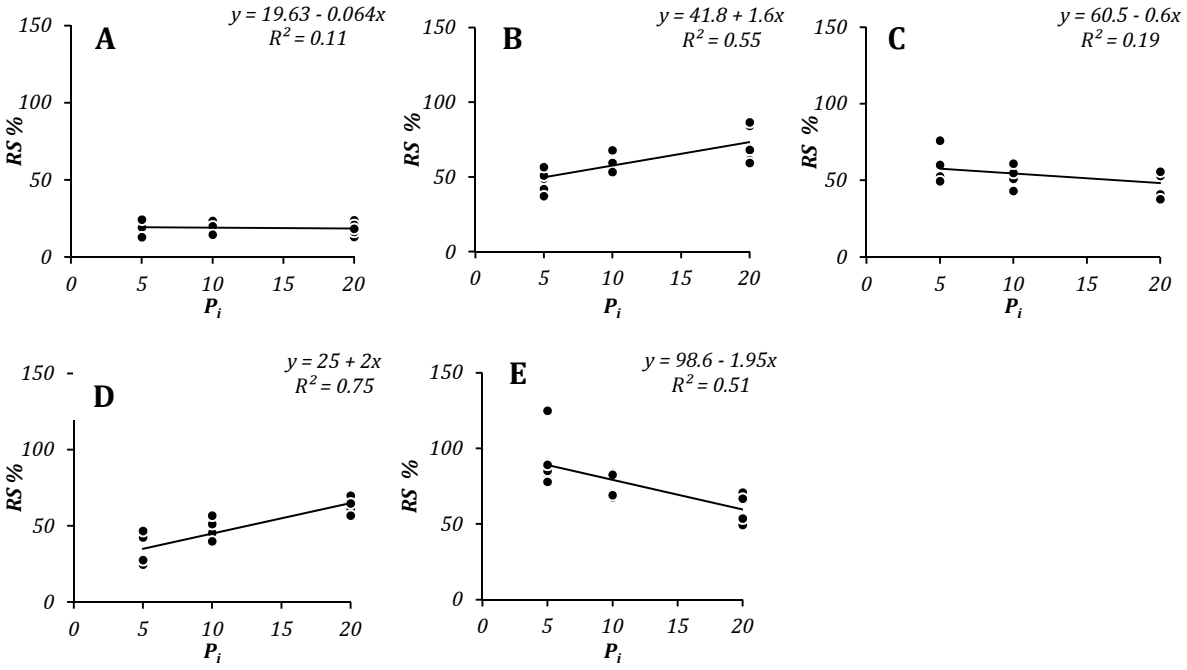

Figure 3. Relationship between initial population densities P_i (eggs+J2/ml soil) of *Heterodera avenae* and relative susceptibility (*RS*) of wheat cultivars: 'Aus 10894' **(A)**; 'Gemmeza 9' **(B)**; 'Iskamish K-2-Light' **(C)**; 'Sahl 1' **(D)**; 'Sakha 93' **(E)**.

Effect of increasing the initial population density of *H. avenae* on grain yield of wheat cultivars

A negative correlation between initial population densities (P_i) of *H. avenae* and the grain yield of wheat cultivars was observed **(Figure 4)**. As the P_i of *H. avenae* increased, the grain yield of all the tested cultivars decreased **(Table 2)**. Reduction in grain yield of the cultivar 'Aus 10894' was not significant at all P_i levels compared to the non infested control and ranged between 4 – 11%. Grain yield of the cultivars 'Capa', 'Gemmeza 9' and 'Sahl 1' was significantly reduced at all P_i levels.

The highest reduction in grain yield (55%) was recorded at a P_i of 20 J2/ml soil on the cultivar 'Capa' followed by the cultivars 'Gemmeza 9' and 'Sahl 1' with reduction of 40 and 39 %, respectively. At a P_i of 5 and 10 J2/ml soil, reduction in grain yield of

cultivars 'Iskamish K-2-Light' (12-18%) and 'Sakha 93' (16-20%) was not significant compared to the non infested control, while the reduction in grain yield was significant at P_i of 20 J2/ml soil.

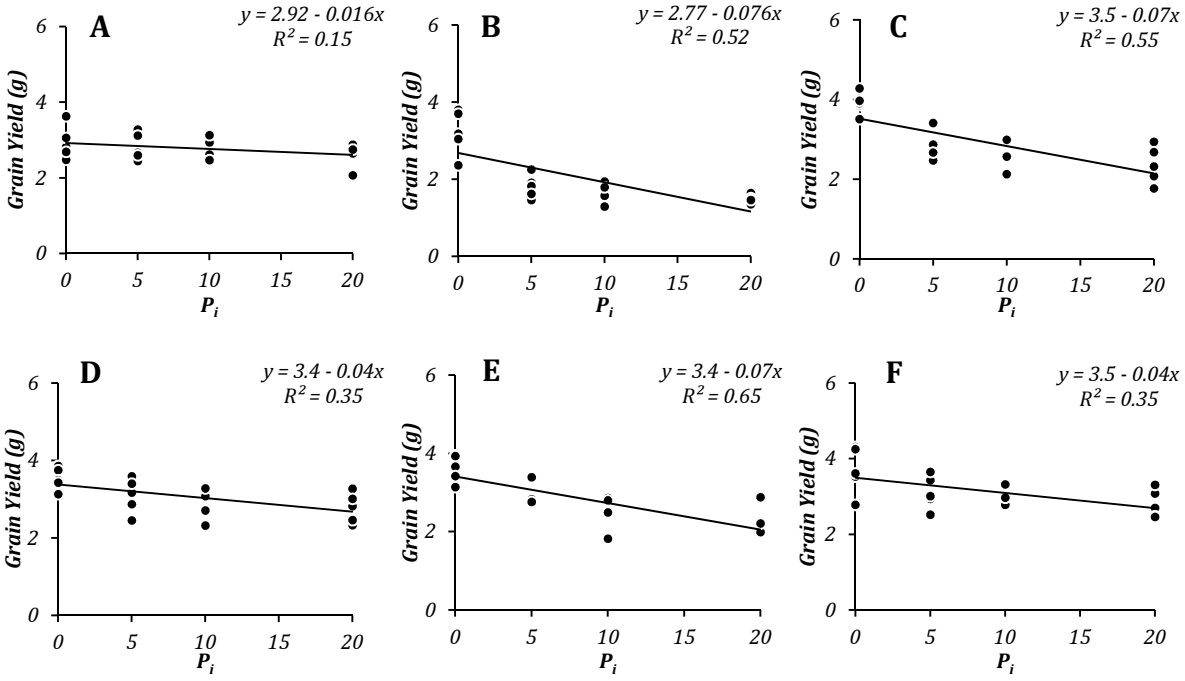

Figure 4. Relationship between initial population densities P_i (eggs+J2/ml soil) of *Heterodera avenae* and grain yield (g) of wheat cultivars: 'Aus 10894' **(A)**; 'Capa' **(B)**; 'Gemmeza 9' **(C)**; 'Iskamish K-2-Light' **(D)**; 'Sahl 1' **(E)**; 'Sakha 93' **(F)**.

Table 2. Effect of initial population densities (P_i) of *Heterodera avenae* on grain yield of wheat cultivars.

Cultivars	**0**	P_i = **5** J2/ml soil		P_i = **10** J2/ml soil		P_i = **20** J2/ml soil	
	Yield (g)[a]	**Yield (g)**	***Red* (%)** [b]	**Yield (g)**	***Red* (%)**	**Yield (g)**	***Red* (%)**
Aus 10894	2.9 ± 0.4 **a**	2.8 ± 0.5 **a**	**03.8**	2.8 ± 0.4 **a**	**05.9**	2.6 ± 0.3 **a**	**11.0**
Capa	3.2 ± 0.6 **a**	1.8 ± 0.3 **b**	**43.7**	1.6 ± 0.3 **b**	**51.1**	1.5 ± 0.3 **b**	**54.7**
Gemmeza 9	3.9 ± 0.3 **a**	2.8 ± 0.4 **b**	**27.4**	2.6 ± 0.4 **b**	**34.3**	2.4 ± 0.5 **b**	**39.6**
Iskamish K-2	3.5 ± 0.5 **a**	3.1 ± 0.5 **ab**	**12.3**	2.9 ± 0.4 **ab**	**18.2**	2.8 ± 0.4 **b**	**21.4**
Sahl 1	3.6 ± 0.4 **a**	2.9 ± 0.3 **b**	**19.0**	2.5 ± 0.4 **bc**	**31.1**	2.2 ± 0.4 **c**	**39.3**
Sakha 93	3.7 ± 0.6 **a**	3.1 ± 0.4 **ab**	**15.9**	3.0 ± 0.2 **ab**	**19.9**	2.8 ± 0.4 **b**	**24.1**

[a] Yield (g)= Means of grain yield/pot ± standard deviation.

Means in a row followed by the same letter are not significantly different based on Tukey test (P ≤ 0.05).

[b] *Red* (%) = Percentage of reduction in grain yield compared to control (0).

Red (%)=((*CP-IP*)/*CP*)*100, where *Red* (%)= percentage of reduction, *CP*= grain yield of control plant, *IP*= grain yield of infested plant.

The tested wheat cultivars showed different degrees of tolerance at nematode P_i levels **(Figure 5)**. The wheat cultivar 'Sakha 93' was the most tolerant cultivar at all P_i levels as the tolerance index (*TI*) ranged between 0.2-0.3. Wheat cultivars 'Iskamish K-2-Light' and 'Aus 10894' were less tolerance and their *TI* ranged between 0.3-0.4 and 0.3-0.5, respectively. Tolerance of wheat cultivars 'Capa' and 'Gemmeza 9' was low to all *H. avenae* P_i levels, as *TI* ranged between 0.5-0.6 and 0.5-0.8, respectively. The lowest tolerance to the nematode was recorded by the cultivar 'Sahl 1' and *TI* ranged between 0.6–0.9.

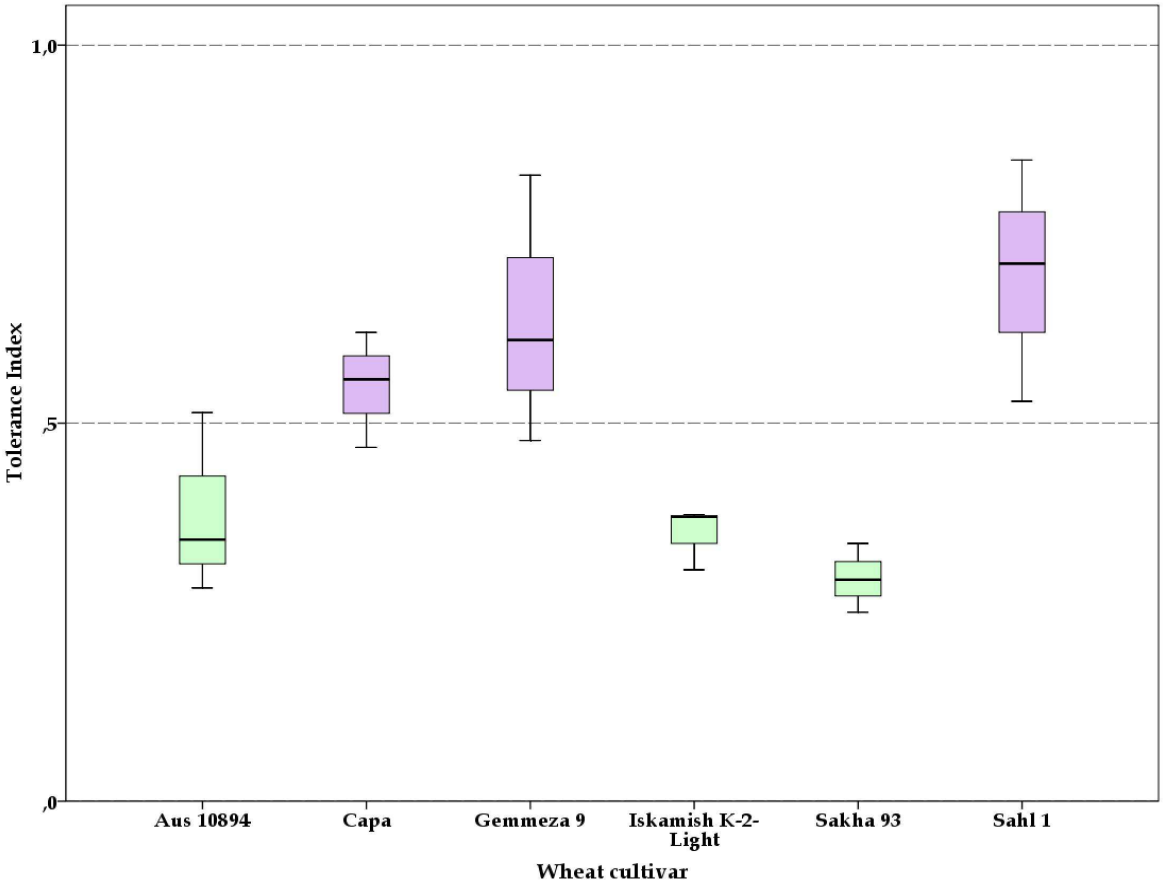

Figure 5. Box plot of the tolerance index of wheat cultivars to initial population densities of *Heterodera avenae*. Tolerance index (*TI*) = ((*GCP-GIP*)/*GCP*)*100/P_f, where, *GCP*= grain yield of control plant, *GIP*= grain yield of infested plant, P_f= final population density of J2/ml soil Tolerance ranking: tolerant (0-0.5); less tolerant (0.5-1) and sensitive (>1).

Effect of increasing initial population density of *H. avenae* on spike weight of wheat cultivars

Regression analyses showed that spike weight of tested wheat cultivars was negatively correlated with initial population densities (P_i) of *H. avenae* **(Figure 6)**. As the P_i of *H. avenae* increased, spike weight of all cultivars decreased **(Table 3)**. Reduction in the spike weight of cultivar 'Aus 10894' was not significant at all P_i levels compared to the non infested control and ranged between 10 – 23%. The highest reduction in spike weight was recorded at a P_i of 20 J2/ml soil on cultivar 'Capa' with 49% reduction followed by the cultivars 'Gemmeza 9' and 'Sahl 1' with reduction of 43 and 44%, respectively. Reduction in spike weight of 'Iskamish K-2-Light' (18-29%) and 'Sakha 93' (17-27%) was not significant at a P_i of 5 and 10 J2/ml soil. Spike weight of the cultivars 'Iskamish K-2-Light' and 'Sakha 93' was significantly reduced at a P_i of 20 J2/ml soil compared to the non infested control. Spike weight of the cultivars 'Capa', 'Gemmeza 9' and 'Sahl 1' was significantly reduced at all P_i levels.

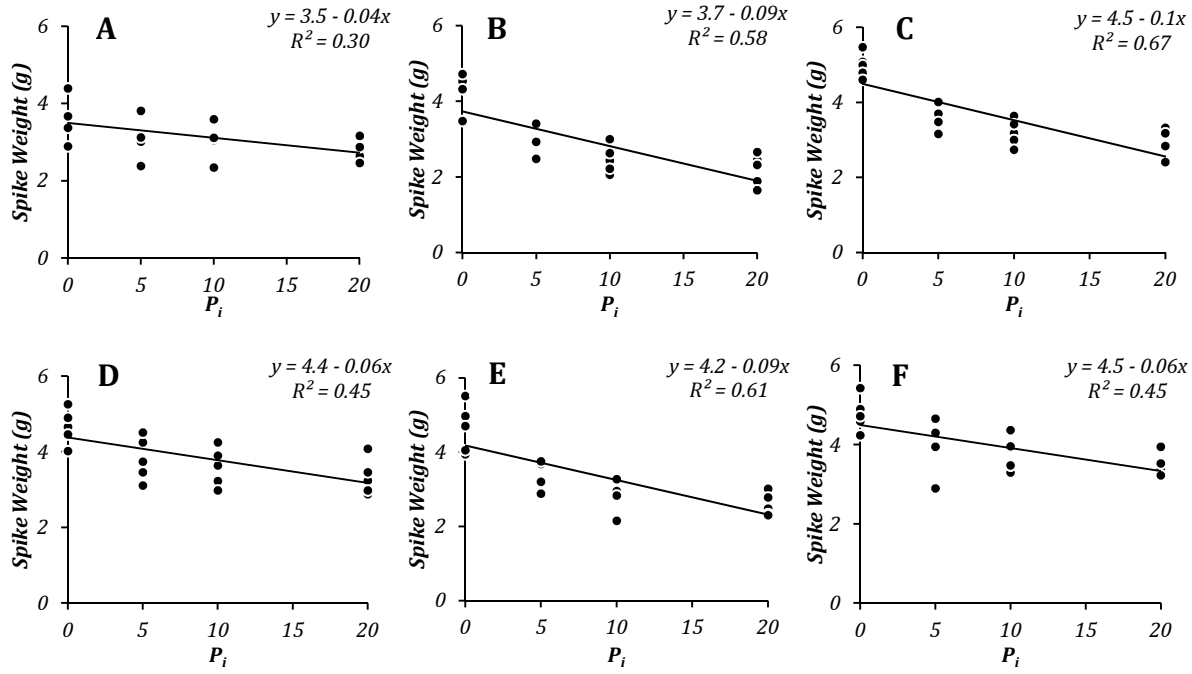

Figure 6. Relationship between initial population densities P_i (eggs+J2/ml soil) of *Heterodera avenae* and spike weight (g) of wheat cultivars: 'Aus 10894' **(A)**; 'Capa' **(B)**; 'Gemmeza 9' **(C)**; 'Iskamish K-2-Light' **(D)**; 'Sahl 1' **(E)**; 'Sakha 93' **(F)**.

Table 3. Effect of initial population densities of *Heterodera avenae* on spike weight of wheat cultivars.

Cultivars	**0**	P_i = **5** J2/ml soil		P_i = **10** J2/ml soil		P_i = **20** J2/ml soil	
	Spike (g)[a]	**Spike (g)**	***Red* (%)** [b]	**Spike (g)**	***Red* (%)**	**Spike (g)**	***Red* (%)**
Aus 10894	3.6 ± 0.5 **a**	3.2 ± 0.6 **a**	**10.3**	3.0 ± 0.4 **a**	**15.3**	2.8 ± 0.3 **a**	**22.5**
Capa	4.3 ± 0.5 **a**	2.8 ± 0.4 **b**	**35.7**	2.5 ± 0.6 **b**	**42.5**	2.2 ± 0.4 **b**	**48.9**
Gemmeza 9	5.0 ± 0.3 **a**	3.6 ± 0.7 **b**	**28.4**	3.2 ± 0.4 **bc**	**35.9**	2.8 ± 0.4 **c**	**43.2**
Iskamish K-2	4.7 ± 0.5 **a**	3.8 ± 0.6 **ab**	**18.2**	3.6 ± 0.5 **b**	**22.8**	3.3 ± 0.5 **b**	**28.6**
Sahl 1	4.6 ± 0.7 **a**	3.3 ± 0.4 **b**	**27.9**	2.9 ± 0.5 **b**	**37.4**	2.6 ± 0.6 **b**	**44.3**
Sakha 93	4.8 ± 0.9 **a**	4.0 ± 0.7 **ab**	**17.2**	3.7 ± 0.4 **b**	**22.2**	3.5 ± 0.3 **b**	**26.9**

[a] Spike (g) = Means of spike weight/pot ± standard deviation.
Means in a row followed by the same letter are not significantly different based on Tukey test (P ≤ 0.05).
[b] *Red* (%) = Percentage of reduction in the spike weight compared to control (0).
Red (%) = ((*CP*-*IP*)/*CP*)*100, where *Red* (%) = percentage of reduction, *CP*= spike weight of control plant, *IP*= spike weight of infested plant.

Effect of increasing initial population density of *H. avenae* on shoot dry weight of wheat cultivars

A negative relationship between the initial population densities (P_i) of *H. avenae* and shoot dry weight of all tested wheat cultivars was recorded **(Figure 7)**. Shoot dry weight of tested wheat cultivars decreased significantly in response to increasing nematode P_i except on the cultivar 'Aus 10894' at a P_i of 5 J2/ml soil **(Table 4**). The highest reduction in shoot dry weight was recorded on the cultivars 'Capa', 'Gemmeza 9' and 'Sahl 1'. The reduction in shoot dry weight of cultivar 'Iskamish K-2-Light' ranged between 25-37%. The lowest reduction in shoot dry weight was recorded on the cultivars 'Aus 10894' and 'Sakha 93' and ranged between 19-28 and 19-34%, respectively.

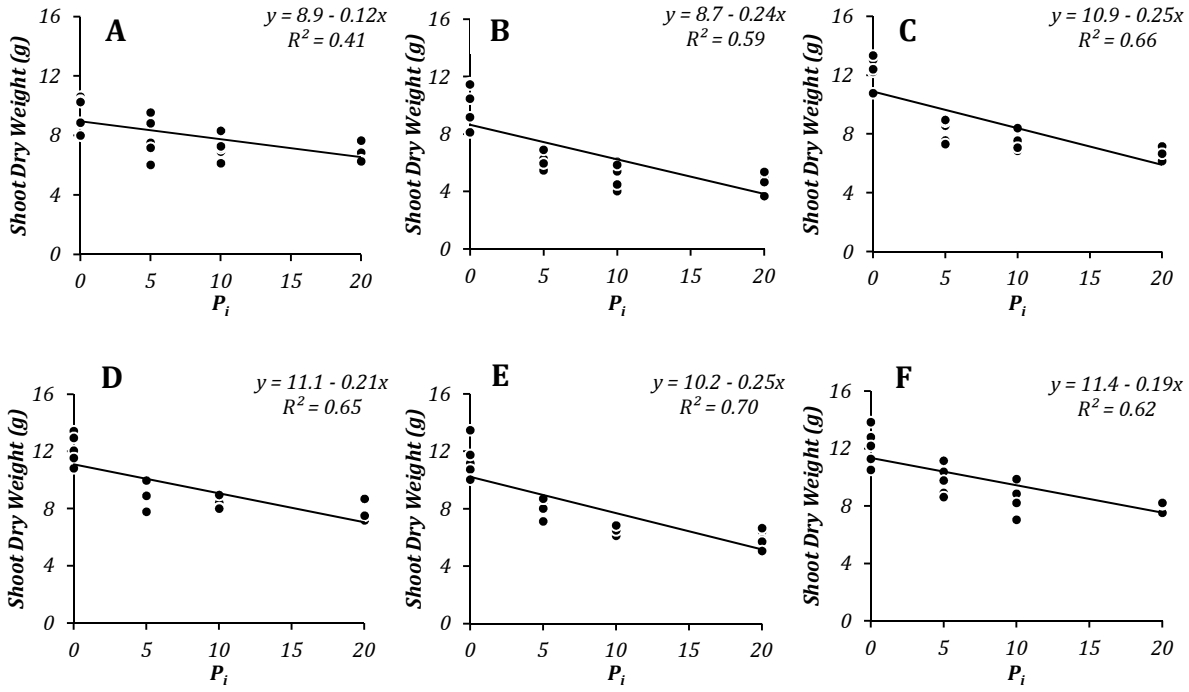

Figure 7. Relationship between initial population densities P_i (eggs+J2/ml soil) of *Heterodera avenae* and shoot dry weight (g) of wheat cultivars: 'Aus 10894' **(A)**; 'Capa' **(B)**; 'Gemmeza 9' **(C)**; 'Iskamish K-2-Light' **(D)**; 'Sahl 1' **(E)**; 'Sakha 93' **(F)**.

Table 4. Effect of initial population densities of *Heterodera avenae* on shoot dry weight of wheat cultivars.

Cultivars	**0**	P_i = **5** J2/ml soil		P_i = **10** J2/ml soil		P_i = **20** J2/ml soil	
	Shoot (g) [a]	**Shoot (g)**	***Red* (%)** [b]	**Shoot (g)**	***Red* (%)**	**Shoot (g)**	***Red* (%)**
Aus 10894	09.6 ± 1.1 **a**	7.8 ± 1.4 **ab**	**18.7**	7.2 ± 0.8 **b**	**25.5**	7.0 ± 1.4 **b**	**27.7**
Capa	10.2 ± 1.5 **a**	6.1 ± 0.5 **b**	**39.7**	5.2 ± 1.3 **b**	**49.0**	4.7 ± 1.0 **b**	**54.0**
Gemmeza 9	12.4 ± 1.0 **a**	8.3 ± 1.5 **b**	**33.2**	7.5 ± 0.6 **bc**	**39.2**	6.7 ± 0.7 **c**	**45.9**
Iskamish K-2	12.2 ± 1.0 **a**	9.1 ± 0.9 **b**	**25.0**	8.3 ± 0.4 **bc**	**31.4**	7.6 ± 0.6 **c**	**37.2**
Sahl 1	11.4 ± 1.3 **a**	8.1 ± 0.6 **b**	**29.2**	6.6 ± 2.1 **c**	**42.5**	5.9 ± 1.2 **c**	**48.0**
Sakha 93	12.1 ± 1.3 **a**	9.8 ± 1.8 **b**	**19.4**	8.8 ± 1.2 **b**	**27.9**	8.0 ± 0.3 **b**	**33.9**

[a] Shoot (g) = Means of shoot dry weight ± standard deviation.
Means in a row followed by the same letter are not significantly different based on Tukey test (P ≤ 0.05).
[b] *Red* (%) = Percentage of reduction in the soot dry weight compared to control (0).
Red (%) = ((*CP-IP)/CP*)*100, where *Red* (%) = percentage of reduction, *CP*= shoot dry weight of control plant, *IP*= shoot dry weight of infested plant.

Effect of increasing initial population density of *H. avenae* on root dry weight of wheat cultivars

Regression analyses showed that root dry weight of the tested wheat cultivars was negatively correlated with initial population densities (P_i) of *H. avenae* **(Figure 8)**. As the P_i of *H. avenae* increased, root dry weight decreased **(Table 5)**. The reduction in root dry weight of the cultivar 'Aus 10894' was not significant at all P_i levels compared to the non infested control and the reduction ranged between 5 – 15%. At a P_i of 5 and 10 J2/ml soil, the reduction in root dry weight of cultivars 'Iskamish K-2-Light' (13-19%) and 'Sakha 93' (14-16%) was not significant, while it was significant at a P_i of 20 J2/ml soil compared to the non infested control. Root dry weight of the cultivars 'Capa', 'Gemmeza 9' and 'Sahl 1' was significantly reduced at all P_i levels. The highest reduction was recorded at a P_i of 20 J2/ml soil on the cultivar 'Capa' with 37% reduction followed by the cultivars 'Gemmeza 9' and 'Sahl 1' with 34 and 32%, respectively.

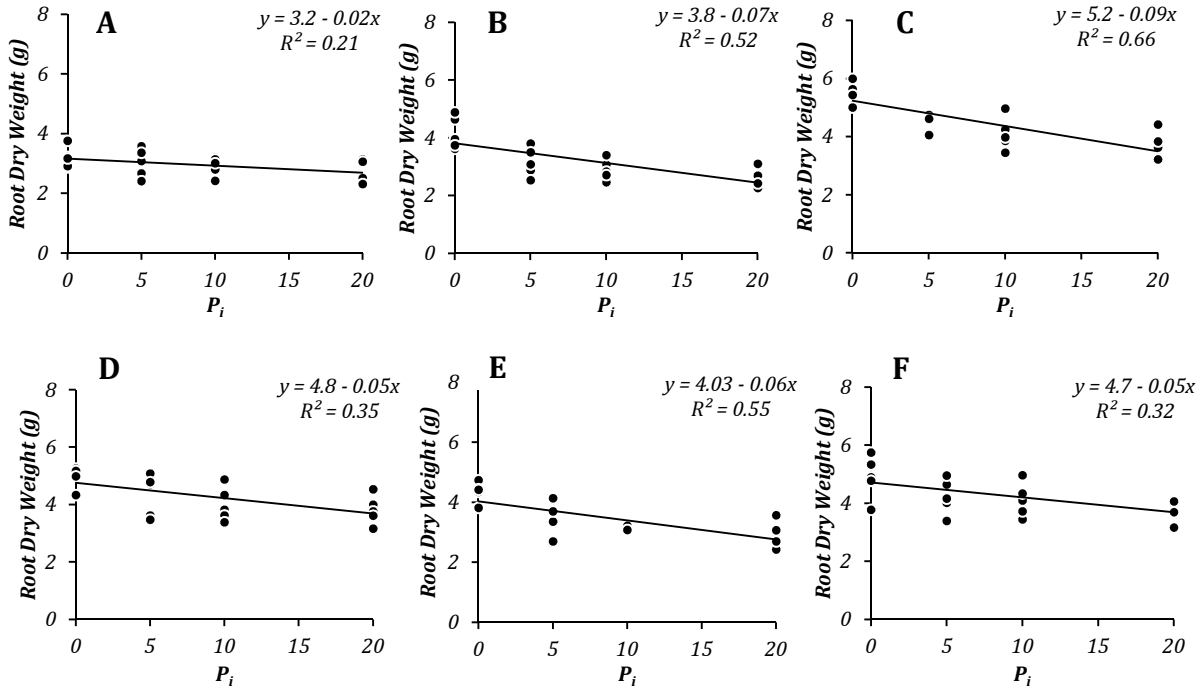

Figure 8. Relationship between the initial population densities P_i (eggs+J2/ml soil) of *Heterodera avenae* and root dry weight (g) of wheat cultivars: 'Aus 10894' **(A)**; 'Capa' **(B)**; 'Gemmeza 9' **(C)**; 'Iskamish K-2-Light' **(D)**; 'Sahl 1' **(E)**; 'Sakha 93' **(F)**.

Table 5. Effect of initial population densities of *Heterodera avenae* on root dry weight of wheat cultivars.

Cultivars	**0**	P_i = **5** J2/ml soil		P_i = **10** J2/ml soil		P_i = **20** J2/ml soil	
	Root (g) [a]	**Root (g)**	***Red* (%)** [b]	**Root (g)**	***Red* (%)**	**Root (g)**	***Red* (%)**
Aus 10894	3.2 ± 0.9 **a**	3.0 ± 1.0 **a**	**05.2**	2.9 ± 0.7 **a**	**08.6**	2.7 ± 0.5 **a**	**15.2**
Capa	4.2 ± 1.1 **a**	3.2 ± 0.9 **b**	**24.2**	2.9 ± 1.1 **b**	**30.5**	2.6 ± 0.7 **b**	**36.6**
Gemmeza 9	5.5 ± 1.9 **a**	4.6 ± 0.8 **b**	**17.0**	4.1 ± 1.7 **bc**	**25.8**	3.7 ± 1.0 **c**	**33.5**
Iskamish K-2	5.0 ± 1.5 **a**	4.3 ± 0.7 **ab**	**12.5**	4.0 ± 1.1 **ab**	**19.1**	3.8 ± 0.8 **b**	**23.0**
Sahl 1	4.4 ± 1.0 **a**	3.5 ± 1.3 **b**	**20.6**	3.1 ± 0.7 **b**	**27.8**	3.0 ± 1.0 **b**	**32.2**
Sakha 93	4.9 ± 0.7 **a**	4.2 ± 1.5 **ab**	**13.6**	4.1 ± 0.6 **ab**	**16.1**	3.8 ± 0.8 **b**	**23.1**

[a] Root (g) = Means of root dry weight ± standard deviation.

Means in a row followed by the same letter are not significantly different based on Tukey test ($P \leq 0.05$).

[b] *Red* (%) = Percentage of reduction in the root dry weight compared to control (0).

Red (%) = ((*CP-IP)/CP*)*100, where *Red* (%) = percentage of reduction, *CP*= root dry weight of control plant, *IP*= root dry weight of infested plant.

DISCUSSION

The nematode damage to host plants depends upon nematode population density in the soil as well as its reproduction in the host plant **(Seinhorst, 1965; Barker and Olthof, 1976)**. A positive correlation between final population densities and increasing initial population densities of *H. avenae* was observed in this experiment. On the other hand, nematode reproduction was negatively correlated with increasing initial population densities. This could be attributed to the competition for feeding sites and the greater damage of infected roots with increasing nematode initial density, which decreases the suitable area of the roots for nematodes to infect, establish and reproduce **(Fisher and Hancock, 1991)**. These results are in accordance with the previous report of **Magi (1989)** who found that the final number of eggs and juveniles increases with increasing initial density but the reproductive rate decreases. Studies on the relationship between initial population densities of *H. avenae* and nematode reproduction on wheat and barley showed significant negative correlations **(Dhawan and Nagesh, 1987; Rivoal and Sarr, 1988)**. **Fisher and Hancock (1991)** reported that the reproduction factor of *H. avenae* reproduced tenfold at low initial densities, while it decreased with increasing in initial population densities of *H. avenae*.

The relative susceptibility of the tested wheat cultivars at a P_i of 5 J2/ml soil was comparable to their relative susceptibility in the previous experiment **(Chapter 4)**. In this study, increasing the initial population densities of *H. avenae* led to increase in the relative susceptibility of the wheat cultivars 'Gemmeza 9' and 'Sahl 1' **(Table 1)**. At a P_i of 5 J2/ml soil, these cultivars were moderately susceptible, while they were susceptible at a P_i of 20 J2/ml soil. This increase in susceptibility may due to the significant increase in the final population density of *H. avenae* on these cultivars following the increase in P_i.

On the other hand, increasing the initial population densities of *H. avenae* led to decrease in the relative susceptibility of the wheat cultivar 'Iskamish K-2-Light' **(Table 1)**. At a P_i of 5 J2/ml soil, this cultivar was susceptible, while it was moderate susceptible at a P_i of 20 J2/ml soil. This decrease in susceptibility may due to the constancy in the final population density of *H. avenae* on this cultivar in spite of the increase in P_i.

Negative relationship between the initial population density and different plant growth parameters (grain yield, spike weight, shoot dry weight and root dry weight) was detected in this study. Previous reports have concluded that losses in the grain yield of wheat caused by *H. avenae* are mainly due to the reduction of the number of spikes, number and weight of grains/spike **(Goent, 1982; Romero *et al.,* 1988; Romero *et al.,* 1991; Zancada and Althofer, 1994)**.

This study indicates that Egyptian populations of *H. avenae* are serious pests of Egyptian wheat cultivars and potentially a limiting factor in the production of wheat in Egypt. The reduction in the grain yield of the Egyptian cultivars by *H. avenae* ranged between 16 - 40% under greenhouse conditions. The substantial reduction in the grain yield of the Egyptian wheat cultivars found in this study indicates that even the lowest P_i (5 eggs and J2/ml soil) caused significant damage to wheat under greenhouse conditions. On the other hand, the local wheat cultivar 'Sakha 93' showed some degree tolerance as the reduction in grain yield was not significant in spite of the high relative susceptibility to *H. avenae* at a P_i of 5 and 10 J2/ml soil. The grain yield of 'Sakha 93' was only significantly reduced at a P_i of 20 eggs and J2/ml soil.

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CHAPTER 6

Main findings and general discussion

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Main Findings:

1- Cereal cyst nematodes were found in five out of seven surveyed sites in Ismailia province and west Sinai, Egypt.

2- All cereal cyst nematode populations collected from Egypt were identified as *Heterodera avenae* based on morphology, RFLP and rDNA-ITS sequence analyses.

3- No differences in ITS-RFLP patterns were detected among the Egyptian populations; however the Egyptian populations could be distinguished from German populations of *H. avenae* (Grafenreuth).

4- The hatching pattern of the Egyptian populations of *H. avenae* was similar to the Mediterranean ecotypes with winter activity while the German population was similar to the Northern ecotypes with spring activity.

5- The *H. avenae* populations from Egypt are of the same virulence phenotype as pathotype Ha13 while the German population could be assigned to pathotype Ha11.

6- None of the tested Egyptian local wheat cultivars was resistant to the Egyptian populations of *H. avenae.*

7- The local wheat cultivar 'Sakha 93' could be classified as tolerant to *H. avenae* populations under greenhouse conditions.

General Discussion:

The cereal cyst nematode (CCN) *Heterodera avenae* Wollenweber, were found in five out of seven surveyed sites in Ismailia province and west Sinai, Egypt; with a frequency of occurrence of 79.4%. This is the first report detecting *H. avenae* infecting wheat in Ismailia and Sinai. Higher prevalence of *H. avenae* was recorded in this study compared to the previous report of **Ibrahim and Handoo (2007)**, who found *H. avenae* infecting wheat fields in Nile Delta at an incidence of 38%. This may be due to the fact that *H. avenae* populations can increase more in light well-draining soils of Ismailia and Sinai than the heavy soil of Nile valley area. The same observation was recorded previously by **Brown (1984)** in South Australia.

Cereal cyst nematodes populations found infecting wheat fields in different regions of Ismailia and west Sinai were identified as a *H. avenae* based on morphology **(Mulvey and Golden, 1983; Lamberti and Taylor, 1986; Sharma and Sharma, 1998)**. No differences in ITS-RFLP patterns were detected among the Egyptian populations; however the Egyptian populations could be distinguished from a German population of *H. avenae* (Grafenreuth).

The Egyptian populations of *H. avenae* belong to *H. avenae* populations Type B according to **Subbotin *et al.,* (2003)**. Similar results were recorded in nearby countries: Israel **(Zheng *et al.,* 2000)**; Iran **(Ahmadi and Tanha Maafi, 2009)**; China **(Peng *et al.,* 2009)**; India **(Subbotin *et al.,* 1999)** and Turkey **(Imren *et al.,* 2012)**. The analyses of ITS region sequences confirmed the species identification of the Egyptian populations, and clustered with *H. avenae* populations from Iran, Saudi Arabia, India, Israel and China.

The Egyptian populations of *H. avenae* were subjected to different temperature and storage periods to determine their ecotype and temperature requirements. The hatching pattern of the Egyptian populations did not differ significantly. The temperature and other agro-ecological factors in the surveyed regions of Ismailia are not distinctly different.

The hatching pattern of the Egyptian populations of *H. avenae* is similar to the Mediterranean ecotype which has winter activity, such as *H. avenae* populations from southern France **(Rivoal, 1978)**, Italy **(Greco, 1981)**, Spain **(Valdeolivas and Romero, 1986)**, South Australia **(Banyer and Fischer, 1971)** and Israel **(Mor *et al.,* 1992)**. Control strategies such as early planting and rotation that are effective against the Mediterranean ecotype of *H. avenae* in southern France and Spain **(Romero *et al.,* 1991; Rivoal and Cook, 1993)** could be applied or developed for Egyptian production systems.

The virulence of *Heterodera avenae* populations from Egypt was characterized on a number of discriminating wheat cultivars from an International Test Assortment. The Egyptian populations of *H. avenae* could be assigned to pathotype Ha13. This pathotype has been reported from Australia **(Brown & Meagher, 1970**; **O'Brien and Fisher, 1979)**. The Egyptian local cultivar 'Sakha 93' was the only wheat cultivar that showed tolerance under greenhouse conditions to all *H. avenae* populations. The same local cultivar was previously reported as a high yielding cultivar with tolerance to water stress **(El-Ashry and El-Kholy, 2005; Ibrahim *et al.,* 2011)**.

No resistance in any of the tested wheat cultivars to the Egyptian populations has been detected in this study. In order to control pathotype Ha13 which is present in Egypt, germplasm with specific resistance genes against this pathotype (like *Cre3* and *Cre6*) have to be deployed **(Ogbonnaya *et al.,* 2001)**. Ideally such resistance is then incorporated into locally adapted cultivars for used in integrated *H. avenae* management programs.

This study also assessed the relation between the nematode initial population density of *H. avenae* and nematode reproduction on different wheat cultivars. Positive correlation between final population densities and increasing initial population densities of *H. avenae* was observed. On the other hand, nematode reproduction was negatively correlated with increasing initial population densities. This could be attributed to the competition for feeding sites and the greater damage of infected roots

with increasing nematode initial density, which decreases the suitable area of the roots for nematodes to infect, establish and reproduce **(Fisher and Hancock, 1991)**.

Negative relationship between the initial population density and different plant growth parameters (Grain yield, spike weight, shoot dry weight and root dry weight) was detected in this study. Previous reports of **Goent (1982); Romero *et al.,* (1988); Romero *et al.,* (1991); Zancada and Althofer, (1994)** have concluded the same negative relation.

This study indicated that *H. avenae* is a serious pest and potentially a limiting factor in the production of wheat in Egypt. To minimize the detrimental effect of cereal cyst nematode on wheat production in Egypt, more information concerning the occurrence and distribution of cereal cyst nematode in wheat growing areas in Egypt, is needed. This research has to be complemented by the following measures:

- Adoption of appropriate farm hygiene and other phytosanitary measures to avoid accelerating the spread of cereal cyst nematode to non infested regions.
- Pathotype characterization of the detected populations by testing for virulence against a number of cereal cultivars from the International Test Assortment for pathotypes definition.
- Introduce resistant germplasm to the pathotype present in Egypt.
- Screening for resistance and tolerance among Egyptian wheat cultivars for further use in breeding programs.
- Development of integrated control programs for cereal cyst nematode including the use of resistant cultivars.

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Curriculum Vitae

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CARRIER OBJECTIVE

Seeking to become a better and knowledgeable Nematologist who is at par with the latest technologies and advancements in agricultural field. I am looking forward in establishing myself in the field of agriculture, to demonstrate my knowledge and technical skills that will contribute to the development and better understanding of science for benefit.

RESEARCH INTEREST

Study the etiology and diagnosis of plant diseases caused by plant-parasitic nematodes on agricultural and horticultural crop plants.

Determine the spatial distribution of the plant-parasitic nematode in the field and monitor nematode population development during the growing season.

Morphological and molecular identification of the economically most important group of plant-parasitic nematodes (especially Root-knot nematode and cereal cyst nematodes). Study of intraspecific variation and the use of new technologies for the development of species identification methods.

Estimate yield loss estimates attributed to nematode infections and develop means to reduced nematode numbers to levels that are not injurious to a specific crop.

Improve agricultural production systems which are affected with yield-reducing plant-parasitic nematodes using economical, environmentally and ecologically safe management techniques.

EMPLOYMENTS

(July 2012 - present) Volunteer researcher in Institute for National and International Plant Health (AG), Julius Kühn-Institut, Braunschweig, Germany.

(December 2011 - Juni 2012) Research assistant in the scientific project 'Forschungsvorhaben zur Biologie von Zystennematoden' in Institute for National and International Plant Health (AG), Julius Kühn-Institut, Braunschweig, Germany.

(September 2009 - November 2011) Volunteer researcher in Institute for National and International Plant Health (AG), Julius Kühn-Institut, Braunschweig, Germany.

(January 2009 - August 2009) Volunteer researcher in Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für Nematologie und Wirbeltierkunde, Münster, Germany.

(December 2004 - December 2009) Assistant Lecturer in Agricultural Botany Department, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt.

(October 1999 - November 2004) Research assistant in Agricultural Botany Department, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt.

ACADEMIC CAREER

M.Sc. (2004) in Agricultural science (Plant-parasitic nematodes) under title "Pathological and biological studies on nematodes infecting fruit trees" from Faculty of Agriculture, Suez Canal University, Egypt.

B.Sc. (1999) in the science of plant protection from Faculty of Agriculture, Suez Canal University, Egypt.

METHODS EXPERIENCE

- Well versed with methods of sampling, extraction, cultivation and morphological identification of plant-parasitic nematodes.

- Basic Microbiology techniques including: preparation of media, buffers and other solutions, culturing of bacteria and Fungi, preparation of cell-extracts, DNA extraction from bacteria, fungi, soil and plant materials.

- Molecular and biochemical techniques for identification, study of genetic diversity and phylogeny of nematodes, including methods of DNA extraction from nematodes, PCR, RFLP, DNA cloning, sequencing and electrophoresis.

- Exceptional knowledge in estimating and screening pathogenicity and host-parasite relationships of plant-parasitic nematode diseases.

- Profound knowledge of methods of chemical control, organic amendments, plant extracts, trapping plants and biological controls of plant parasitic nematodes by and antagonistic microorganisms.

ADDITIONAL SKILLS

- Team work competence with local/international research groups.

- I have experience in preparing and equipping scientific laboratories as I participated in creating the Nematology laboratory in Institute for National and International Plant Health (AG), Julius Kühn-Institut, Braunschweig, Germany.

- Knowledge of research methodologies, data and information mining, molecular data analysis, writing and presenting reports.

- Competent knowledge for PC computer systems (International Computer Driving License (ICDL)), familiar with Microsoft Word, Excel, PowerPoint, Sigma Plot, Analyst, GenStat, Gel compare I and II, Adobe Photoshop, Basic local Alignment Search Tool, internet and more.

- Good knowledge in most of the statistical analysis programs (SPSS, SASS, COSTAT and others).

- Competent written and spoken English language skills (TOEFL Internet-Based Test (IBT) with Score 70) and good knowledge in basics German language (Deutsch GIII (9 levels - 400 hours)).

TRAINING COURSES

- I performed a training workshop during the period January 21st – 23th, 2009, in "Identification of plant-parasitic nematodes". The workshop was performed in Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für Nematologie und Wirbeltierkunde, Münster, Germany.

- I performed a training workshop during the period April 9st – 13th, 2006, in the area of "Risk Assessment, Environmental Modeling and Life Cycle Analysis". The workshop was performed within the framework of the Tempus- Meda Programme sponsored by European Commission.

- I performed a one month training at the INCA laboratory of Marghera (Venice; Italy), Environmental Microbiology Division, during the period June 1st – 30th, 2005, under the scientific supervision of Dr. Fulvio Zecchini.

- I gained a deep practical experience by doing advanced practical courses in my master's degree in plant diseases caused by nematode, plant resistance to nematode diseases, environment and

diseased spread, physiology of parasitism, economics and Statistics, design and analysis of the agricultural experiments, Report and Seminar preparing, as well as, different computer courses. These courses provided an opportunity for me to gain experience with plant-nematode interaction, microscopic analysis, data management and statistical processing, creation of scientific publications and presentations.

- Through my B.Sc. studying history I have taken practical courses in plant protection, pest control, plant pathology, microbiology, genetics, soil science, analytical chemistry, chemistry of pesticides and biological control. These practical courses gave me a good understanding of how to deal with nematode and plant pathogens using different microscopic and molecular techniques, chemical and biological control techniques.

TEACHING

- I contribute in the teaching of the practical part (as a Assistant Lecturer in the period from October 1999 to December 2009 in the Faculty of Agriculture, Suez Canal University) of the following courses: plant parasitic nematode diseases, principal of microbiology, principal of plant pathology, crops diseases, fruit trees diseases and plant diseases.

PUBLICATIONS

- **Baklawa, M., Nasr, S. and Massoud, S. (2011).** *Plant-parasitic nematodes infecting fruit trees in Egypt.* VDM Verlag Dr. Müller GmbH&Co. KG, Saarbrücken, Germany. pp181. ISBN: 978-3-639-32791-5. (Book)

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- **Baklawa, M., Massoud, S. and Nasr, S. (2005).** Seasonal fluctuation of plant-parasitic nematodes associated with five fruit crops in Ismailia governorate. Proceeding of the 6th Arabian Conference for Horticulture, Ismailia, Egypt.

- **Baklawa, M., Massoud, S. and Nasr, S. (2005).** Survey of plant-parasitic nematodes associated with five fruit crops in Ismailia governorate. Proceeding of the 6th Arabian Conference for Horticulture, Ismailia, Egypt.

- **Baklawa, M. (2004).** Pathological and biological studies on nematodes infecting fruit trees. M.Sc. Thesis, Fac. Agric. Suez Canal Univ., 181pp.

CONFERENCE CONTRIBUTIONS

- **Baklawa, M., Niere, B. and Massoud, S. (2012).** Variation in reproduction and damage potential of Egyptian populations of *Heterodera avenae* on different wheat varieties. (Oral presentation) 31st International European Society of Nematologists Symposium, 23rd - 27th September, Adana, Turkey.
- **Baklawa, M., Niere, B. and Massoud, S. (2012).** Cereal cyst nematodes on wheat in Ismailia, Egypt: Occurrence, morphometrics and molecular characterization. (Oral presentation) The third Workshop of the International Cereal Cyst Nematode Initiative. 21-23th September 2012. Adana, Turkey.
- **Baklawa, M., EL-Kady, G. and Massoud, S. (2012).** Using SCAR-PCR techniques to identify root-knot nematode infecting Ismailia orchards, Egypt. (Poster) 31st International European Society of Nematologists Symposium, 23rd - 27th September, Adana, Turkey.
- **Baklawa, M., Niere, B. and Massoud, S. (2012).** Damage potential of different initial population densities of *Heterodera avenae* from Egypt on wheat varieties. (Poster) 58th Deutsche Pflanzenschutztagung "Pflanzenschutz – alternativlos", 10-14 September, Braunschweig, Germany.
- **Baklawa, M., Niere, B. and Massoud, S. (2012).** Influence of temperature and storage periods on the hatching behavior of *Heterodera avenae* from Egypt. (Oral presentation) 64th International Symposium on Crop Protection, Gent, Belgium.
- **Baklawa, M., Niere, B., Heuer, H. and Massoud, S. (2012).** Morphological and molecular characterization of *Heterodera avenae* populations from Egypt. (Poster) Annual meeting of the DPG Nematology working group, Berlin, Germany.
- **Baklawa, M., Niere, B. and Massoud, S. (2011).** Damage and reproduction potentials of Egyptian populations of *Heterodera avenae* on wheat in Ismailia, Egypt. (Oral presentation) Annual meeting of the DPG Nematology working group Ak-free living nematodes, Wageningen, Netherlands.
- **Baklawa, M., Massoud, S. and Niere, B. (2009).** Occurrence of cereal cyst nematodes (*Heterodera* spp.) in wheat fields in Ismailia Governorate, Egypt. (Poster) Tropentag 2009-Biophysical and Socio-economic frame conditions for the sustainable management of natural resources, University of Hamburg, Germany.
- **Baklawa, M., Massoud, S. and G. EL-Kady (2009).** Identification of root-knot nematode species infecting banana and grape orchards in Ismailia Governorate, Egypt. (Poster) Tropentag 2009-Biophysical and Socio-economic frame conditions for the sustainable management of natural resources, University of Hamburg, Germany.
- **Baklawa, M., Massoud, S. and Nasr, S. (2005).** Seasonal fluctuation of plant-parasitic nematodes associated with five fruit crops in Ismailia governorate. (Oral presentation) The 6th Arabian Conference for Horticulture, Ismailia, Egypt.

- **Baklawa, M., Massoud, S. and Nasr, S. (2005).** Survey of plant-parasitic nematodes associated with five fruit crops in Ismailia governorate. (Oral presentation) The 6th Arabian Conference for Horticulture, Ismailia, Egypt.

MEMBERSHIP IN THE PROFESSIONAL SCIENTIFIC SOCIETIES

- Member in European Society of Nematologists (ESN) (http://www.esn-online.org/).

- Locally, member in the Egyptian Society of Agricultural Research, Egyptian Society of Agricultural Nematology, Egyptian Society of Biological Control, Egyptian Society of Plant Pathology, Egyptian Society of Pest Control and Environmental protection.

HONORS AND AWARDS

- First prize in the Best Student Poster competition during the 31st International Symposium of the European Society of Nematologists, held in Adana (Turkey) in September 2012 (http://www.esn-online.org/esn-2012-turkey). The title of the poster was 'Using SCAR-PCR techniques to identify root-knot nematodes infecting Ismailia orchards, Egypt'.

- Scientific Excellence Award from Suez Canal Authorization and Port Said governorate in 2006 for the scientific achievement in the Master thesis 'Pathological and biological studies on nematodes infecting fruit trees'.

- Appreciation Award from Suez Canal University in 2005 for the general excellence in the Master Thesis 'Pathological and biological studies on nematodes infecting fruit trees'.

REFERENCES

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